XVIII

Physiological Zoölogy

OCTOBER, 1945

Seasonal Alterations of Growth Rate in Chimeric Feathers of Bronze-Saddle Gene in the Bronze Leghorn Capon . . . . . *Hilo Wang* 335

Flocking Behavior and the Social Hierarchy in Small Flocks of White Leghorns . . . . . *A. M. Guhl, W. E. Collias, and W. C. Allee* 365

Sexual Mounting Behavior in the Male Rat: Effects of Castration and Hormone Administration . . . . . *Frank A. Beach* 390

Behavior of the Brooding Provided by the Male Cichlid Fish, *Tilapia macrocephala*, on the Spawning Frequency of the Female . . . . . *Lester R. Aronson* 403

Ecological Relationships in the Genus *Tetrahymena* . . . . . *George W. Kidder, C. A. Stuart, Virginia G. McGinnis, and Virginia C. Dewey* 415

Thiamine Deficiency as a Factor Limiting Growth of the Ciliate *Tetrahymena geleii* . . . . . *K. P. Hall and W. B. Cowpert* 445

Errata in Physiological Zoölogy 451

C. Brooks and Marjorie Mohammadan Brooks, The Permeability of Living Cells, *M. H. Jacobs*.—Hans Schaefer, Elektrophysiologie, Vol. I: Allgemeine Elektrophysiologie; Vol. II: Spezielle Elektrophysiologie, *Frank A. Brown, Jr.* 457

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EXPERIMENTAL ALTERATION OF GROWTH RATE IN CHIMAERIC FEATHERS OF BREAST-SADDLE ORIGIN IN THE BROWN LEGHORN CAPON[1]

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THE PROBLEM

It is well known that the rate of feather regeneration is a property of the tract in which it is located. In the male and castrate of the Brown Leghorn fowl, for instance, feathers of the breast and saddle (hereafter to be abbreviated as "B" and "S," respectively) grow at specifically different rates (Juhn and Gustavson, 1930; Juhn, Faulkner, and Gustavson, 1931; Lillie and Juhn, 1932). These rates are 1.70 and 1.47 mm. (p. 337) per day, respectively, for the B and S. That they are inherent in the epidermal cells of these tracts and, therefore, constitute part of the tract specificity has been demonstrated by Wang (1943). He found that the specific rates of growth remained unaltered when the dermal portion of the papilla was interchanged from one tract to the other.

An interesting situation consequently presents itself when B and S cells are combined to form the epidermal component of a single papilla (Lillie and Wang, 1941, 1943, 1944; Wang, 1943). In such a case it is evident that, if each type of epidermis were to maintain its own rate of regeneration, bending of the feather would result. But all feather-chimaerae, regardless of their derivation, are free from curvature of any kind. This fact alone indicates that a feather-chimaera grows at a uniform rate (Lillie and Wang, 1943), although the problem has not been yet fully analyzed. The purpose of the present investigation is to ascertain the nature of this uniform growth rate. Can it be that of B or of S alone and unmodified, or is it a compromise between the two?

This work started from our observations that nearly all feather-chimaerae are intermediate in length and have an apex pointed to an angle somewhere between those characteristic of the two tracts. We noticed, too, that chimaeric feathers required a longer period of regeneration. Our interest in the problem was revived when we recently found that the introduction of hashed saddle papillae into a host (B) papilla would also result in a chimaeric feather considerably

[1] This research was aided by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago. A preliminary report was published in the *Anatomical Record* (Wang, 1944*b*).

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longer than a normal B feather. Serious efforts were then begun in the hope of determining whether the observed lengthening of chimaeric feathers is accompanied by reduction in the growth rate of the faster partner, B. In a series of experiments, comparable portions of B and S papillae were grafted in different ways to either whole or parts of B papillae. The resulting feathers were measured throughout their regeneration at regular intervals in order to provide a substantial basis for direct comparison of B and S effects. Feathers which showed no evidence of incorporation of the grafts served as additional controls.

ANALYSIS OF DATA

All experiments were done on Brown Leghorn capons. The results to be presented belong to three groups:

1. Feather-chimaerae whose morphogenetic implications have been rather fully analyzed in previous publications. These feathers, 184 in all, comprise a large portion of our collection of feather-chimaerae. They include feathers from combinations of sagittal and frontal half-papillae (Lillie and Wang, 1941, 1943) and from grafted sectors of papillae (Lillie and Wang, 1944).

2. Feathers derived from B papillae incorporating ventral grafts made of hashed S papillae of three kinds: (*a*) moderately hashed dorsal halves, (*b*) severely hashed dorsal halves, and (*c*) hashed ventral halves.

3. The results of two groups of experiments in which all feathers were measured at 4-day intervals, beginning on the twenty-fourth day after operation and continuing until complete regeneration. In the first group, comparison was made between ventral halves of B and S papillae grafted similarly to whole B papillae and ventral B half-papillae grafted to whole S papillae. In the second group, combinations of two ventral B half-papillae, fused face to face, were contrasted with similar combinations between B and S ventral half-papillae. The purpose of the latter group was to produce tuft feathers (rhachis-free feathers; see Lillie and Wang, 1941) of two distinct kinds—the homologous and the heterologous, respectively. Graphs, plotted on the basis of the measurements of length, are presented.

A. EVIDENCE FROM FEATHER-CHIMAERAE

A feather-chimaera is either a single or a twin feather bearing readily discernible morphological features of B and S (Wang, 1943, p. 328), which owe their origin to the epidermis of their respective tracts incorporated within the same papilla. It can be produced in a variety of ways; the amount and, still more important, the location (loci) of the composite cells both before and after the operation determine the morphological types of the resulting chimaerae. Strictly speaking, the most typical chimaerae are those derived from combinations of half-papillae, either sagittal or frontal. The five types of chimaerae so obtained are listed in Table 1. They are the most important with regard to the question of growth rate, for in them the two kinds of epidermal cells are present in approximately equal numbers.

It is necessary for us to adopt a set of standard values of growth rate of normal B and S feathers to which we will refer for comparison with the observed experimental values. The controls of the specific experiments, while fulfilling their particular needs, are, however, not adequate for this purpose, on account of their lack of uniformity. Calculations are based on Lillie (1940: Tables 1 and 2 for breast, and Tables 3 and 4 for saddle feathers). A distinction is made between average growth rate and maximal growth

rate. The former represents the average daily increment during the entire period of regeneration, i.e., total length attained by the feather divided by the total number of days. The maximal rate is the highest rate represented by the straight part of the growth curve (days 13–40 for an average of 3 anterior B feathers, 13–34 for that of 3 posterior B feathers, and 13–61 in one and 14–61 in the other measurements of S feathers by Lillie). This computation has given the following values in millimeters of growth per day: *Average growth rate:* anterior B = 1.58, posterior B = 1.82 (the mean, 1.70 mm. per day gives an indication of the average growth rate of B feathers in general); S = 1.47. *Maximal growth rate:* anterior B = 2.00, posterior B = 2.50 (an average of 2.25); S = 1.78. For a check, the mean of average growth rate of 49 B feathers used as controls is calculated;[2]

TABLE 1

TOTAL LENGTH (IN MM.) OF FEATHER-CHIMAERAE*

Type	No.	Generations				Average Length
		First	Second	Third	Fourth	
Bilateral	60:1	130	.....	.....	.....	136.0
	60:4	141	148	.....	.....	
	31:2	133	140†	131	128	
Longitudinal	89:8	120	127	129	.....	126.5
	89:9	135	137	137	137	
	301:8	118†	133	140	.....	
	302:10	115	.....	.....	.....	
	303:8	118	128	.....	.....	
	303:9	122	122	.....	.....	
	303:10	110	125	.....	.....	
Transverse	89:1	122†	127	129	.....	124.0
	91:4	124	124	.....	.....	
	301:2	101	117	117	.....	
	301:3	115	120	124	.....	
	304:1	139	145	147	.....	
	304:5	117	122	119	.....	
Pseudo-bilateral	90:1	120	118	123	123	124.0
	90:2	123	126†	127	122	
	90:3	.....	114	120	114	
	90:9	148	147	146	.....	
	304:7	115	127	128	.....	
	304:8	113†	130	130	.....	
	304:9	106	114	120	.....	
Twins	89:3	150	152†	148	.....	141.0
	89:7	159	158	160	.....	
	90:4	143	138	137	.....	
	301:5	110	117	125	.....	
Mean.....	.....	125	130	132	125	130.0

* All feather-chimaerae herewith presented (except the bilateral chimaerae) are from Lillie and Wang (1943). Of these bilateral chimaerae, No. 31:2 is shown in Lillie and Wang (1941, Fig. 7), while the remaining two have not been previously reported.

† Illustrated in previous publications (Lillie and Wang, 1941, 1943).

[2] Three feathers (Table 3, B) give 1.78; 18 feathers (Table 5), 1.79; 10 feathers (Table 6), 1.75; 14 feathers (Table 7), 1.76; 1.52 and 1.48 for controls No. 1 and No. 2, respectively (Table 8); 1.55 and

this gives a value of 1.75 mm. per day. The deviation of 0.05 mm. above the standard value calculated from Lillie's data possibly results from the fact that the majority of the controls were either middle or posterior B feathers. Despite this difference, we will select 1.70 mm. per day as the standard average growth rate for B feathers in all future references. It will contrast with a similar value of 1.47 mm. per day for S feathers.

Similarly, some standard values should be adopted for considerations on length. Inasmuch as the gradients of length within the tracts are present in no less degree than those of growth rate, we need to measure a large number of normal feathers for each tract. Measurement of a total of 207 S and 189 B feathers from 6 capons has shown the average length of these feathers to be 143.6 and 101.8 mm., respectively. These values likewise serve as a basis for comparison.

Table 1 gives 76 measurements of total length of feathers produced in four successive generations of a total of 27 feather-chimaerae. All of them are conspicuously longer than B feathers, with some approaching the S feather in length. This is true regardless of the derivation, and the lengths remain much the same throughout the recorded generations.[3] The significance of such aspects will be understood in connection with the growth rate of these feathers.

Growth rate and length of 10 of these chimaerae have already been briefly treated (Lillie and Wang, 1943, Table 6, pp. 15–16). By comparing their per diem increment, i.e., during the straight part of the total curves of growth, it was shown that they grew at a rate somewhere between the maximal rates of growth of the two tracts. It was further pointed out that such an intermediate rate holds only for the longitudinal (B/S) chimaerae in which the B and S cells grow simultaneously at all loci of the collar. In the case of the transverse (S/B) chimaerae, however, typical S and B rates were demonstrated to accompany the respective S (apical) and B (basal) parts.

We will now examine the data on 14 of these 27 chimaerae further.[4] These feathers were measured for one or two consecutive generations at 3–4-day intervals, beginning on the thirteenth day, and were plucked promptly upon complete regeneration (Table 2). The feathers as a whole are somewhere between typical B and S values in regeneration time, total length, and growth rate. The latter ranges from 1.48 mm. (No. 90:3) to 1.67 mm. (Nos. 304:8 and 89:3) per day—rates which are, respectively, slightly above the S and below the B rate of growth.

These feathers evidently have deviated from the theoretical intermediate B and S growth rate, which is 1.585 mm. per day, the mean of the standard B and S rates (p. 337). Thus, out of the 14 chimaerae, 10 deviate in the S direction, having an average rate of less than 1.585, while only 4 (Nos. 89:9, 304:7, 304:8, and 304:9) deviate in the B direction, with an average rate of above 1.585 mm. As a result, the mean (1.55) of all these

1.57 for controls No. 1 and No. 2, respectively (Table 9).

[3] The slight increase in the average total length shown by the second- and third-generation feathers is not statistically significant. The shorter first-generation feathers, commonly observed for all operated follicles of this kind, are believed to reflect the unfavorable conditions resulting from the operation.

[4] Measurement of Nos. 304: 5, 301: 8, 90: 2, 89: 9, 301: 3, 304: 7, 304: 8, 304: 9, and 90: 3 was presented before (Lillie and Wang, 1943); but, except for the first three of these feathers, the data here used are based on the measurement of a different generation. Nos. 31: 2, 89: 8, 89: 3, 89: 7, and 403: 1 have not been previously used, while No. 90: 1, which was used in the early work, is not presented here.

chimaerae is 5.4 per cent higher than the average rate for S and 10 per cent lower than that of B. Similarly, a closer adherence to the length of the slower partner is manifested. Thus, 132.8 mm. is approximately 8 per cent less than the standard S average length (143.6) and about 30 per cent more than the similar value for B (101.8). On the assumption that the numbers of B and S epidermal cells are equal at the time of combination, this deviation appears to be significant.[5] The possible implication is that the resultant rate represents an interaction between the epidermal cells in which the slower partner (S) has the dominating influence.

The data need to be examined with regard to another relevant point. In Table 2 we have grouped the feather-chimaerae on the basis of derivation. It is revealed that those with B as the dorsal component (B/S) have a higher growth rate than those of the reciprocal combination, i.e., S/B. These rates average, respectively, 1.60 and 1.51 mm. per day. The significance of this difference lies in the fact that the rates represent an increase of 0.05 mm. over the total mean (1.55) for the former and a decrease of 0.04 mm. for the latter. This is an indication that in these feathers the dorsal component of the composite papillae has an influence on the growth rate independent of, and in addition to that of, the general S epidermal cells shown above. Such an influence is to be ascribed

TABLE 2

AVERAGE PER DIEM GROWTH RATE OF SOME OF THE FEATHER-CHIMAERAE LISTED IN TABLE 1

Derivation	No.	Type	Plucking of Two Successive Generations				Regeneration in Days	Feather Length (in Mm.)	Per Diem Rate (in Mm.)	Av. for Types
			Gen.	Date	Gen.	Date				
B-S	31:2	Bilateral	2	Aug. 26	3	Nov. 24	93	140	1.51	.....
B/S	89:8	Longitudinal	2	Aug. 25	3	Nov. 17	84	129	1.54	1.57
	89:9	Longitudinal	3	Nov. 17	4	Feb. 9	84	137	1.63	
	301:8*	Longitudinal	2	Nov. 10	3	Feb. 9	91	140	1.54	
	304:7	Pseudo-bilateral	1	Oct. 1	2	Dec. 18	78	127	1.63	1.65
	304:8	Pseudo-bilateral	1	Oct. 1	2	Dec. 18	78	130	1.67	
	304:9*	Pseudo-bilateral	1	Oct. 1	2	Dec. 9	69	114	1.65	
	89:7	Twin	1	June 9	2	Sept. 17	100	158	1.58	.....
						Mean	83.4	133.5	1.60	.....
S/B	301:3	Transverse	2	Nov. 10	3	Feb. 1	83	124	1.50	1.51
	304:1	Transverse	1	Oct. 1	2	Jan. 4	95	145	1.53	
	304:5*	Transverse	1	Nov. 10	2	Jan. 31	82	122	1.49	
	90:2	Pseudo-bilateral	2	Aug. 25	3	Nov. 17	84	127	1.51	1.49
	90:3*	Pseudo-bilateral	3	Nov. 15	4	Jan. 31	77	114	1.48	
	89:3	Twin	1	May 20	2	Aug. 25	97	152	1.57	.....
						Mean	86.5	130.5	1.51	.....
						Total mean	85.5	132.8	1.55	.....

* Data on measurement of length in Table 3.

[5] This deviation will be considerably greater if we take 1.75 mm. per day (average of 49 controls, p. 337) as the standard mean growth rate of breast feathers.

to the action of the organizing field confined to the dorsal half of the dermal papilla (Lillie and Wang, 1944). Its action has been demonstrated in defective B and S feathers to be proportional to the growth tempo characteristic of the respective tracts (Lillie and Wang, 1944, p. 20). The present analysis shows, therefore, that in combinations of frontal half-papillae, a reduction in growth rate of the more rapidly growing partner is the result to be emphasized; the amount of this reduction varies, however, according to which partner becomes the rhachis-forming component. In other words, we are probably dealing with the combined effect of two separable factors in these feathers (cf. p. 356; also, further discussion on p. 360).

We will now turn our attention to a closer study of 4 of the 14 feathers as representatives of the four distinct types of feather-chimaerae. Each was chosen because its average growth rate is closest to the mean of the respective type. These are: No. 301:8 for longitudinal (B/S) chimaerae, No. 304:5 for transverse (S/B) chimaerae, No. 304:9 for pseudo-bilateral chimaerae of B/S derivation, and No. 90:3 for pseudo-bilateral chimaerae of S/B derivation (Table 2). These feathers were believed to be reasonably free from an effect of gradients operating within the host tract (Juhn, Faulkner, and Gustavson, 1931; Faulkner, 1932; Lillie and Juhn, 1932; Holmes, 1935; Juhn, 1937 and 1938; Fraps and Juhn, 1936; Fraps, 1938), since they occupied similar positions on the breast. This was equally true of the controls, which represented one unoperated follicle nearest to the experimental feather in each of the three experiments (Nos. 301, 304, and 90). For convenience, the complete measurements of length of the chimaerae and of their controls are listed separately in Table 3, A and B, respectively.

The averages of the three controls (Table 3, B) and the four representative feather-chimaerae (Table 3, A) are plotted in Figure 1. These growth curves are very informative, since the relative total length and regeneration period of these feathers can readily be observed and compared. The first thing to be noted is that all four curves of the chimaerae have a less steep slope than that of the control, indicating a reduced growth rate in the feather-chimaerae.[6] Secondly, all four chimaerae are longer and require more time to complete regeneration than the control. These are some general characteristics of the feather-chimaerae. There are also to be considered certain specific differences between the various types of chimaerae. In terms of growth rate, the pseudo-bilateral chimaera of B/S derivation (No. 304:9) exceeds all four: 1.65 mm. (average) and 2.0 mm. (maximal, between days 1–51) per day, as compared to 1.78 and 2.1 (between days 1–39), respectively, for the control. Next came the typical longitudinal chimaera (B/S), No. 301:8, with 1.54 and 1.74 (days 1–70) mm. per day as average and maximal growth rates. The remaining two, both of S/B derivation, are nearly equal in their average growth rate (1.49 for No. 304:5 and 1.48 for No. 90:3) but differ in other important respects, as will be revealed below. In general, therefore, the significance of the origin of the dorsal component (p. 361) in the composite papillae in the determination of growth rate of feather-chimaerae is well illustrated by these representatives.

[6] The different starting-points at the beginning of measurement are due to variations in a delayed emergence, usually characteristic of the experimental feathers.

TABLE 3

MEASUREMENT OF LENGTH OF FOUR REPRESENTATIVE FEATHER-CHIMAERAE WITH CONTROLS

A. FEATHER-CHIMAERAE*

Date of Measurement	Regeneration Age and Length							
	No. 301:8 Longitudinal		No. 304:5 Transverse		No. 304:9 (B/S) Pseudo-bilateral		No. 90:3 (S/B) Pseudo-bilateral	
	Day	Mm.	Day	Mm.	Day	Mm.	Day	Mm.
Oct. 13					13	15.5		
17					17	23.0		
20					20	27.0		
23					23	35.0		
26					26	42.0		
29					29	48.5		
Nov. 1					32	55.5		
4					35	63.5		
7					38	70.0		
10					41	77.0		
13					44	83.0		
17					48	92.5		
21					51	98.0		
23	13	13.0	13	9.0				
24					54	102.5		
27	17	18.5	17	11.0	57	107.0		
28							13	11.5
30	20	23.0	20	15.0	60	110.0		
Dec. 1							16	17.5
3	23	28.5	23	20.0	63	112.0		
5							20	21.0
6	26	33.5	26	25.0	66	113.5		
9	29	40.0	29	30.0	69	114.0	24	28.0
12	32	46.0	32	36.0			27	33.0
15	35	51.0	35	41.0			30	38.0
18	38	57.5	38	47.0			33	43.0
22	42	64.0	42	54.5			37	50.0
25	45	72.0	45	62.0			40	55.0
29	49	80.0	49	71.0			44	61.5
Jan. 1	52	85.5	52	79.5			47	67.5
4	55	93.0	55	86.0			50	74.0
7	58	99.5	58	93.0			53	79.0
10	61	106.0	61	99.0			56	85.0
13	64	111.5	64	105.0			59	91.5
16	67	117.0	67	110.5			62	97.0
19	70	122.0	70	115.5			65	103.0
22	73	129.0	73	118.5			68	107.5
25	76	132.5	76	120.0			71	111.5
28	79	134.0	79	121.0			74	113.0
31	82	136.0	82	122.0			77	114.0
Feb. 3	85	138.0						
6	88	139.5						
9	91	140.0						
Av. growth rate per day (mm.)	1.54		1.49		1.65		1.48	

* Plotted as curves *B–E* in Fig. 1.

TABLE 3—*Continued*

B. CONTROLS

Regeneration Age (in Days)	Length of Feather (in Mm.)			
	Expt. 90	Expt. 304	Expt. 301	Av.*
13	15.5	14.5	18.0	16.0
16	26.0	22.0	25.5	24.5
20	39.0	31.0	35.0	35.0
23	49.0	40.5	42.5	44.0
27	57.0	50.0	52.0	53.0
30	65.5	58.0	58.0	60.5
33	70.5	69.0	67.5	69.0
36	76.0	76.0	73.0	75.0
39	82.5	85.0	81.5	83.0
42	86.0	91.0	85.5	87.5
45	94.0	95.0	90.0	93.0
48	96.5	100.0	96.0	97.5
51	98.0	101.0	101.0	100.0
54	98.5	102.5	102.0	101.0
57	98.5	103.0	103.0	101.5
Average growth rate per day (mm.)				1.78

* Plotted as curve *A* in Fig. 1.

The transverse chimaera (No. 304:5), however, presents certain features not seen in the others, as shown by the fact that its growth curve crosses that of No. 90:3. (The other curves, including that of the control, do not cross one another except occasionally at the terminus, the reason for which is easily understood.) This is obviously because No. 304:5 started with a low rate, which increased gradually until, after the fiftieth day, it had exceeded that of No. 90:3. Thus the growth rate of the transverse chimaerae is such as actually to give the appearance of detectable transitions at certain points along the curve. If one lays a transparent ruler over curve D of Figure 1, he may discover three somewhat clear-cut slopes (without counting the terminal leveling-off, beginning approximately on the seventieth day, since this period of greatly declined growth rate coincides with the formation of the calamus and is hence characteristic of all feathers). The first of these is from day 13 to 26, with a total increment of 16 mm., or an average per diem rate of 1.23 mm.; the second, from day 26 to 42, with a total increment of 29.5 mm., or an average rate of 1.84; the third, from day 42 to 70, in which period the rate has gone up to 2.18 mm. per day, very close to the standard maximal rate for B. Or, if we take days 42–82 as a whole, we get an average per diem rate of 1.69 mm., thus indicating that the feather actually grew at a typical breast rate during this period. These transitions were manifested as much by all of the other three symmetrical transverse chimaerae that were also measured (Nos. 89:1, 301:2, and 301:3). Therefore, they are probably significant. One logical interpretation seems to be that the growth rate of a transverse chimaera changes from time to time in accordance with the cellular composition of the collar (Lillie and Wang, 1943, pp. 9–11), and that the different rates just shown correspond closely, if not exactly, to the pure S rate of its apex, the intermediate

(chimaeric) rate of its middle transitional zone in which the barbs are made of breast tip and saddle base, and the pure B rate of the entire basal portion (Lillie and Wang, 1943, Fig. 9). This suggests that the modification of rate comes about only when both B and S epidermal cells are actually utilized in the collar, since the normal S and B rates are found when the parts of the feather involve the participation of one epidermis alone. From this it is concluded that the intermediate growth rate is the result of the joint action of S epidermal cells on the cells of B in the region of the collar where both kinds of epidermis are simultaneously engaged in active multiplication. When the collar is formed by one epidermis alone, or when both are present but only one of the two is active,[7] the rate ap-

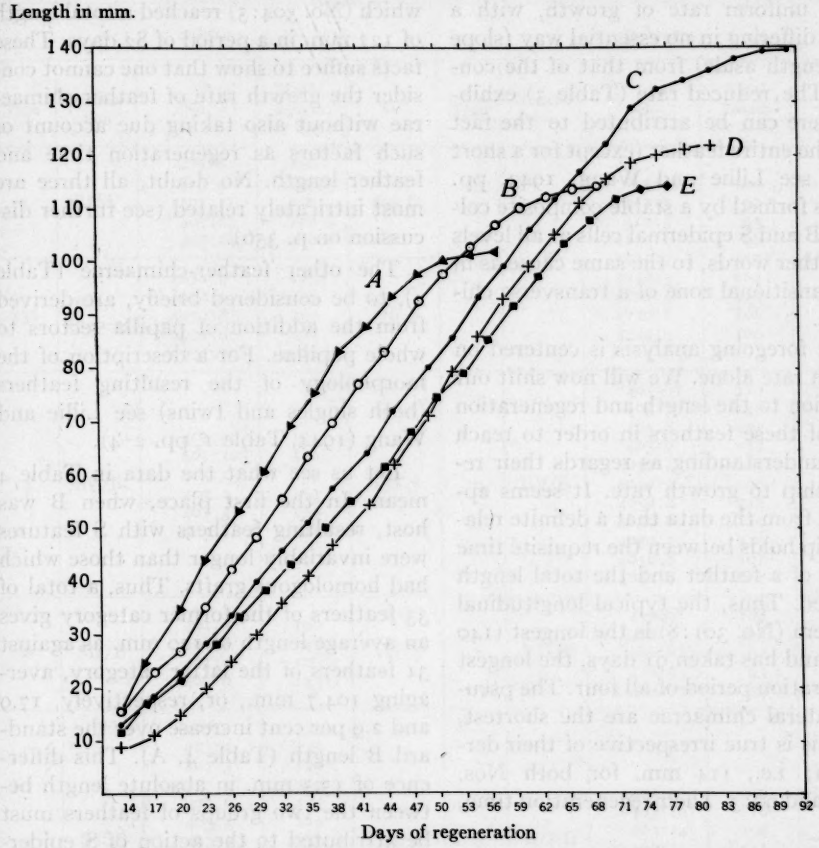

FIG. 1.—Growth curves, based on Table 3, of normal breast feather (*A*) and those of four different types of feather-chimaerae (*B–E*). *A* = control (average of 3 feathers); *B* = pseudo-bilateral chimaera of B/S derivation; *C* = typical longitudinal chimaera; *D* = typical transverse chimaera; *E* = pseudo-bilateral chimaera of S/B derivation.

[7] This statement is based on our original assumption (Lillie and Wang, 1943, p. 9 and Fig. 3) that

pears to be that of the cells involved, either B or S.

The longitudinal chimaerae, on the other hand, as represented by No. 301:8 (Fig. 1, curve *C*), are characterized by a fairly uniform rate of growth, with a curve differing in no essential way (slope and length aside) from that of the control. The reduced rate (Table 3) exhibited here can be attributed to the fact that the entire feather (except for a short apex; see Lillie and Wang, 1943, pp. 6–9) is formed by a stable composite collar of B and S epidermal cells at all levels —in other words, to the same cause as in the transitional zone of a transverse chimaera.

The foregoing analysis is centered on growth rate alone. We will now shift our attention to the length and regeneration time of these feathers in order to reach some understanding as regards their relationship to growth rate. It seems apparent from the data that a definite relationship holds between the requisite time period of a feather and the total length attained. Thus, the typical longitudinal chimaera (No. 301:8) is the longest (140 mm.) and has taken 91 days, the longest regeneration period of all four. The pseudo-bilateral chimaerae are the shortest, and this is true irrespective of their derivation; i.e., 114 mm. for both Nos. 304:9 and 90:3. Their regeneration time, though differing considerably (69 and 77 days, respectively), is nevertheless the shortest of the four. Occupying an intermediate position are the typical transverse chimaerae, the representative of which (No. 304:5) reached a total length of 122 mm. in a period of 82 days. These facts suffice to show that one cannot consider the growth rate of feather-chimaerae without also taking due account of such factors as regeneration time and feather length. No doubt, all three are most intricately related (see further discussion on p. 356).

The other feather-chimaerae (Table 4), to be considered briefly, are derived from the addition of papilla sectors to whole papillae. For a description of the morphology of the resulting feathers (both singles and twins) see Lillie and Wang (1944, Table 1, pp. 2–4).

Let us see what the data in Table 4 mean. In the first place, when B was host, resulting feathers with S features were invariably longer than those which had homologous grafts. Thus, a total of 33 feathers of the former category gives an average length of 120 mm. as against 31 feathers of the latter category, averaging 104.7 mm., or, respectively, 17.9 and 2.9 per cent increase over the standard B length (Table 4, A). This difference of 15.3 mm. in absolute length between the two groups of feathers must be attributed to the action of S epidermal cells. Furthermore, the observed increase in length is characteristic of all chimaeric feathers, irrespective of their manner of origin. The effect may, therefore, be regarded as common to all S epidermal cells in a composite papilla.

In the second place, in contrast to the above, the incorporation of S cells into S papillae yielded feathers showing no appreciable effect of the graft (Table 4, B, left). Here, 16 feathers gave an aver-

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in the collar of a transverse chimaera "the breast cells (occupying ventral positions) invade the saddle area (occupying dorsal position) and eventually completely replace it." Whether the said replacement involves actual displacement of the S cells by B cells, or is more in the sense of some form of B dominance over the S component without cellular replacement, remains to be tested. Further discussion of this question will be found in a forthcoming paper dealing with the response of feather-chimaerae to the action of female hormone (p. 361). What concerns us here is the important fact that, whatever may be the case, the basal part of a transverse chimaera is identical with B-tract specificity in morphology as well as in growth rate.

TABLE 4

TOTAL LENGTH OF FEATHERS DERIVED FROM ALL POSSIBLE BREAST-SADDLE COMBINATIONS (16 TYPES) OF A WHOLE PAPILLA PLUS A MID-DORSAL OR MID-VENTRAL SECTOR*

A. BREAST AS HOST

S Grafts					B Grafts				
Type	Designation	No.	Total Length (in Mm.)	Av.	Type	Designation	No.	Total Length (in Mm.)	Av.
13	B/SV	407:8 407:9 407:11 407:12 407:14	106 104 130 108 116	112.8	9	B/BV	412:1† 412:3 412:5 412*a*:1 412*a*:2	120 97 93 96 110	103.0
13*a*	B/S (3–0–3)	407*a*:2 407*a*:5 407*a*:8† 407*a*:9 407*a*:10	123 113 132 106 109	116.6			412*a*:3 412*a*:4 412*a*:8 412*a*:9	102 100 108 106	
14	SV/B	401:9 401:10† 405:1 405:2 405:3 405:4	121 116 120 114 120 121	118.7	10	BV/B	405:6† 405:7 405:8 405:9 405*a*:1 405*a*:3 405*a*:4 405*a*:6 405*a*:7 405*a*:8 405*a*:9	110 116 120 112 105 98 118 110 110 101 101	109.2
6	SD/B	401:3 401:4 401:5† 401:6	100 145 108 133	121.5	2	BD/B	412:6† 412:7 412:8† 412:9 412:10	108 103 97 93 94	98.8
5	B/SD	407:1 407:2 407:3 407:4† 407:6 403:1 403:2 403:3 403:4 403:5 403:6 403:7 403:8	133 135 119 130 121 129 139 121 132 134 140 123 132	129.8	1	B/BD	403:9 403:10† 403:14 406:1 406:5 406:7	102 110 109 115 105 108	108.0
Grand average				120.0					104.7

* The types of experiments and their designations are those of Table 1 in Lillie and Wang (1944). The measurements of total length herewith presented are made of the first generation of all the feathers except those precociously completed—e.g., Nos. 5 and 6, Expt. 402, and whenever evidence of nonparticipation of the graft was distinctly shown.

† Illustrated in Lillie and Wang (1944).

TABLE 4—*Continued*

B. SADDLE AS HOST

S Grafts					B Grafts				
Type	Designation	No.	Total Length (in Mm.)	Av.	Type	Designation	No.	Total Length (in Mm.)	Av.
3	S/SD	404:10	162	162.0	7	S/BD	404:1 404:2 404:3 404:4 404:5 404:6 404:7 404:8 408:8 408:10†	168 151 159 161 147 170 173 152 173 152	160.6
11	S/SV	413:1† 413:2 412:3 413:4	152 146 132 152	145.5	15	S/BV	408:2 408:3 408:4 408:5	143 143 175 121	145.5
					15*a*	S/B (3–0–3)	517:1 517:2	148 152	150.0
4	SD/S	413:2 413:4	151 153	152.0	8	BD/S	402:3 402:4 409:1 409:2 409:4 409:5	152 156 130 126 120 130	135.7
12	SV/S	409:6 409:7 409:9 409*a*:2 409*a*:3 409*a*:4 409*a*:7 409*a*:8 409*a*:9	147 148 142 140 145 167 170 151 146	150.7	16	BV/S	402:8† 402:9 414:1 414:2 414:4 414:5	160 155 131 124 127 123	138.6
Grand average				152.5					145.3

age length of 152.5 mm., only 6.2 per cent above standard S length. When S papillae incorporated heterologous B grafts (Table 4, B, right), 28 feathers gave an average length of 145.3 mm., nearly normal S length. Such a small difference is not significant, especially when the relative effectiveness of the B and S epidermal cells in modifying feather length is compared (cf. right column of Part B with left column of Part A of Table 4). In view of the close correlation between growth rate and length in their manifestation of the rate-modifying effect of S, as already shown by the feather-chimaerae (p. 340), we would expect the B cells to be capable of shortening the S length as much as the S cells could lengthen the B feather, providing their effects on the reciprocal host were equal. Apparently this is not the case; the effect of S is, by far, the greater.

Our data on the length of the regenerative period of these feather-chimaerae

are limited to a few cases in which the feathers were plucked immediately following complete regeneration. They are: the second generation of No. 410:10 (type 14, SV/B), which required 74 days to complete, or 1.60 mm. per day—an average rate well between the standard B and S average rates. No. 405*a*:9 (type 10, BV/B) completed its second generation in 62 days, or 1.78 mm. per day. All 8 feathers of Experiment No. 203 (type 5, B/SD) completed their second generation in 79 days. Their average length is 131 mm.—hence an average rate of 1.66 mm. per day.

B. EVIDENCE FROM GRAFTING HASHED SADDLE PAPILLAE

The experiments to be described were designed primarily to show whether the organizing properties inherent in the dorsal half of the dermal papilla (Lillie and Wang, 1941, 1944; Wang, 1943) can be restored after complete spatial derangement.[8] Our present interest is confined to the effect of such grafts on the growth rate of the host feathers.

The experimental procedure used is strictly aseptic. A number of dorsal or ventral half-papillae of actively regenerating S feathers were obtained in the usual manner (see Lillie and Wang, 1941, pp. 109–10, for the basic technique). While immersed in Ringer's solution contained in a tiny watch glass, the half-papillae were cut into very tiny bits with scissors, as in preparing embryonic extract, thus reducing the cellular material to a mash of thoroughly mixed dermal and epidermal tissues. In this form it was divided into lots of the size of approximately one-sixth of the volume of a papilla, and each was transferred autoplastically into a longitudinal groove made on the ventral surface of a breast papilla. Not more than 20 follicles were operated upon at one time. For controls, the follicles occupying the same relative positions on the unoperated right breast (for the same reason as explained on p. 340) were tattooed with India ink. These, as well as the operated ones, were frequently observed in order to record the time of their emergence and of their complete regeneration.

In the first of these experiments (Table 5) 30 dorsal S half-papillae furnished 19 such hashed grafts (leaving ample allowance for discard). One of these, No. 6 (not listed), was a failure, i.e., no feather arose from the follicle. Of the remainder, Nos. 3 and 11 gave no sign of incorporation of the graft, while the other 17 feathers were all chimaeric. Numbers 13 and 14 are twin chimaerae. A brief description of the morphology, particularly with reference to the saddle effect, the length in the first and second generations, the duration of regeneration of the second-generation feathers, and the computed average growth rates, is given in Table 5, together with their controls.

The difference between the controls and those feathers which display the unmistakable S marks of reddish, lacy-margined barbs is sufficiently expressed in the relative total length of 100 mm. for the controls, as against 120 mm. for the experimentals. The former required an average of 56 days, as against 73 days for the latter, in completing regeneration. The average growth rates are thus 1.79 and 1.64 mm. per day, respectively.

The S characters of two feathers (Nos. 4 and 10) entirely disappeared in the second generation, evidently as a result of the accidental loss of the incorporated graft. Concurrently the feathers returned to the normal B length and rate of growth. For this reason, these feathers

[8] See Wang (1944*c*) for a preliminary account of the results.

were not used in calculating the average values of the experimental feathers.

The results of this experiment show conclusively that the effect of S epidermis in altering the B rate, as evidenced by the feather-chimaerae, can also be demonstrated by this new method of grafting.

In the second experiment of the series, 18 dorsal S half-papillae supplied 10 similarly prepared grafts except that these were subjected to more severe treatment by subsequently being ground in a tiny glass mortar. The results are listed in Table 6. The epidermal cells in all but one (No. 7) of these grafts evidently failed to survive, since the resulting feathers displayed no S features at all. The after-feathers of most of the feathers are, however, either enlarged or profusely fluffy; but this effect merely reflects the survival of some dermal tissue

TABLE 5

LENGTH, GROWTH RATE, AND MORPHOLOGY OF FEATHERS DERIVED FROM GRAFTING MODERATELY HASHED DORSAL SADDLE HALF-PAPILLAE VENTRALLY TO WHOLE BREAST PAPILLAE (EXPT. 902)

No.	First Generation Length (Mm.)	Second Generation			Control			Morphology*
		Length (Mm.)	Regeneration (Days)	Per Diem Rate (in Mm.)	Length (Mm.)	Regeneration (Days)	Per Diem Rate (in Mm.)	
1.....	115	114	70	1.63	105	59	1.78	Large after-feather with S margin and tips
2.....	135	136	82	1.66	102	57	1.79	Conjoined twin: S right; B with S margin, left
3†.....	99	100	56	1.79	99	54	1.83	Normal B
4.....	120	103	58	1.77	112	63	1.78	Profuse after-feather and fluff with S margin‡
5.....	117	114	70	1.63	103	57	1.79	Bilateral S margin in vane; profuse after-feather
7.....	120	117	72	1.62	98	54	1.81	Face-to-face twin; S partner defective
8.....	108	104	59	1.76	95	53	1.79	Large profuse after-feather, lengthened fluff barbs
9.....	119	113	70	1.61	101	56	1.80	Bilateral S margin in vane; large after-feather
10.....	115	105	60	1.75	97	54	1.80	Bilateral S margin; large profuse after-feather‡
11†.....	96	97	55	1.77	96	54	1.78	Normal B
12.....	130	121	76	1.59	110	62	1.77	Large after-feather and profuse fluff
13.....	144	135	86	1.57	106	59	1.80	Perfect twin of the B/SD type (Lillie and Wang, 1944)
14.....	138	130	81	1.61	111	62	1.79	A twin similar to No. 13
15.....	126	117	71	1.65	108	61	1.77	Bilateral S margin
16.....	134	129	80	1.61	113	64	1.77	Large after-feather and profuse fluff with S margin
17.....	128	122	76	1.61	110	63	1.75	Similar to No. 16
18.....	140	136	86	1.58	115	65	1.77	Unilateral S margin
19.....	123	120	73	1.64	112	63	1.77	Apically conjoined twin; profuse fluff
Mean.	125.5	120	73	1.64	100	56	1.79	

* A feather, unless otherwise stated, has the main feature of a breast feather; its description specifies only the effect of the graft. Feathers marked with † are not used in calculating the averages. See text (p. 347) for reasons.

of the hashed grafts, as was ascertained previously (Lillie and Wang, 1944; Wang, 1944*a*). The only safe criterion used for the survival of S epidermis was the presence of typical lacy S barbs, incorporated anywhere into a host feather. Number 7 is the only one conforming to this requirement. It is a chimaera with an S half-vane on left, is 107 mm. long, and has an average growth rate of 1.60 mm. per day. The other cases do not differ from the controls to any appreciable extent.

The third experiment, likewise, is a demonstration of lack of S effect as the result of failure of incorporation of the grafts. Fourteen grafts were made from 22 S ventral half-papillae which had been reduced to a mash as in the first experiment. None of the resulting feathers (Table 7) carried any S component, although the majority, like those of the last experiment, registered a decided effect on the after-feather or in the profusing and lengthening of fluff barbs. Consequently, it is not necessary to list their morphology in Table 6. The controls and experimental feathers are indistinguishable in both length and growth rate.

The foregoing results leave no doubt that the presence or absence of the effect under test depends solely on whether the S epidermal cells in the hashed grafts succeeded in contributing materially to the resulting feather. The results also tend to confirm the hypothesis (p. 339) that the ability to exert such an effect is present in all cells of the S epidermis and that

TABLE 6

LENGTH, GROWTH RATE, AND MORPHOLOGY OF FEATHERS DERIVED FROM GRAFTING SEVERELY HASHED DORSAL SADDLE HALF-PAPILLAE VENTRALLY TO WHOLE BREAST PAPILLAE (EXPT. 910)

No.	First-Generation Length (Mm.)	Second Generation			Control			Morphology
		Length (Mm.)	Regeneration (Days)	Per Diem Rate (in Mm.)	Length (Mm.)	Regeneration (Days)	Per Diem Rate (in Mm.)	
1.....	92	94	53	1.77	89	51	1.75	Large profuse after-feather, but no S trait
2.....	93	93	52	1.79	92	51	1.80	Similar to No. 1
3.....	85	86	50	1.72	88	51	1.73	Large profuse after-feather with B tips
4.....	86	86	50	1.72	85	51	1.67	Half-vane B; profuse after-feather with no S feature
5.....	91	90	50	1.80	94	52	1.81	"Laddered" half-vane after-feather, all B
6.....	88	87	50	1.74	90	51	1.76	Profuse after-feather and increased barb length in fluff, but no S feature
7.....	105	107	65	1.65	86	50	1.72	Chimaera with S half-vane on left
8.....	92	90	51	1.76	95	53	1.79	Normal B
9.....	89	89	50	1.78	92	52	1.77	Profuse after-feather with B tips
10.....	83	84	49	1.72	88	51	1.73	Similar to No. 9
Mean.	90.4	90.6	52	1.74 or 1.76*	89.9	51.3	1.75	

* Without counting No. 7, which is the only feather manifesting effect of the graft.

it will express itself whenever given the chance.

C. THE CONCLUDING EXPERIMENTAL EVIDENCE

Our final evidence has come from two series of experiments. The first series is a repetition of type 13*a* in Lillie and Wang (1944), i.e., the addition of a whole ventral half-papilla to a host papilla. Two capons were used: in one, 10 B papillae received a ventral S half-papilla ventrally in each; in the other, 10 follicles of comparable sites in the breast received homologous half-papillae as controls. Two feathers from normal follicles located close to the middle of the operated regions were measured, along with the experimental feathers, as controls of the respective experiments. The results are presented in Tables 8 and 9, respectively.

Of the 10 heterologous combinations, Nos. 1, 3, 6, 8, 9, and 10 gave rise to chimaeric feathers; the remaining 4 combinations were normal B feathers without a trace of S character. Hence, the latter are grouped with the 2 controls. This is justified since there is no difference whatsoever between these cases and the controls (Table 8, B). We will now consider the differences between the experimental and control groups. While the controls required an average of 60 days to reach a length of 93.0 mm. (an average rate of 1.55 mm. per day), the chimaeric feathers increased their period of regeneration to 80 days and attained a length of 107.0 mm, thus giving a much lower average rate of 1.34 mm. per day. These differences are well shown by curves *A* and *B*, respectively, in Figure 2.

If we compare the regeneration rate in the straight part of the curves, the difference between the homologous and heterologous groups is equally decisive. In the former (Table 9, Fig. 3) the two curves representing the experimental and control feathers are practically parallel

TABLE 7

TOTAL LENGTH AND GROWTH RATE OF FEATHERS DERIVED FROM GRAFTING HASHED VENTRAL SADDLE HALF-PAPILLAE TO WHOLE BREAST PAPILLAE. NONE OF THE FEATHERS SHOWS ANY TRACE OF SADDLE FEATURE (EXPT. 911)

No.	First-Generation Length (Mm.)	Second Generation			Control		
		Length (Mm.)	Regeneration (Days)	Per Diem Rate (in Mm.)	Length (Mm.)	Regeneration (Days)	Per Diem Rate (in Mm.)
1	99	100	59	1.70	101	59	1.71
2	103	100	59	1.70	99	59	1.68
3	107	105	59	1.78	104	59	1.76
4	102	101	59	1.71	102	59	1.73
5	107	104	59	1.76	105	59	1.78
6	103	102	59	1.73	103	59	1.75
7	107	105	59	1.78	107	59	1.81
8	109	107	59	1.81	105	59	1.78
9	103	103	59	1.75	104	59	1.76
10	101	101	59	1.71	102	59	1.73
11	110	108	60	1.80	108	60	1.80
12	106	104	59	1.76	105	59	1.78
13	113	113	63	1.80	111	62	1.79
14	110	110	62	1.78	108	60	1.80
Mean	105	104.5	60	1.75	104.6	59.5	1.76

TABLE 8

MEASUREMENT (IN MM.) OF FIRST-GENERATION FEATHERS DERIVED FROM GRAFTING VENTRAL HALVES OF SADDLE PAPILLAE VENTRALLY TO WHOLE PAPILLAE OF THE BREAST (EXPT. 930)

Days of Regeneration	A. Those That Gave Evidence of Incorporation of the Graft							B. Those That Failed To Incorporate the Graft						
	No. 1	No. 3	No. 6	No. 8	No. 9	No. 10	Av.*	No. 2	No. 4	No. 5	No. 7	Control No. 1	Control No. 2	Av.*
24.....	16.0	15.0	20.0	12.5	14.0	18.5	16.0	26.0	26.5	31.0	21.5	25.0	26.0	26.0
28.....	22.0	21.5	25.5	18.0	19.5	25.5	22.0	33.0	35.5	42.5	32.0	36.0	35.0	36.0
32.....	27.0	25.5	32.5	25.0	27.0	31.0	28.0	43.0	46.5	53.0	43.5	47.0	46.0	46.5
36.....	34.5	33.5	39.0	31.5	34.5	37.0	35.0	52.5	58.0	62.5	53.0	57.0	56.0	56.5
40.....	44.0	41.5	46.5	40.0	44.0	42.0	43.0	59.5	67.5	73.0	62.5	67.5	65.0	66.0
44.....	51.0	48.0	52.5	48.5	52.0	48.0	50.0	68.5	75.5	82.0	73.0	76.0	73.0	75.0
48.....	59.5	57.5	62.5	55.5	62.0	57.0	59.0	74.5	86.0	92.0	84.5	85.0	82.0	84.0
52.....	68.5	65.0	71.5	66.0	71.0	66.0	68.0	85.0	89.5	98.0	87.5	89.5	84.5	89.0
56.....	79.0	74.5	80.0	74.5	79.0	75.0	77.0	90.0	90.5	102.0	89.5	90.0	88.0	92.0
60.....	87.0	81.0	88.0	81.5	85.5	81.0	84.0	90.0	94.5	103.0	89.5	91.0	89.0	93.0
64.....	93.5	88.5	95.0	87.0	94.0	88.0	91.0	.....	.....	.....	.....	.....	.....	.....
68.....	102.0	95.5	101.0	93.5	101.5	95.0	98.0	.....	.....	.....	.....	.....	.....	.....
72.....	107.0	101.0	106.5	98.5	108.0	97.0	103.0	.....	.....	.....	.....	.....	.....	.....
76.....	110.0	104.0	108.5	100.5	111.0	102.0	106.0	.....	.....	.....	.....	.....	.....	.....
80.....	113.0	105.5	109.5	101.0	113.0	102.0	107.0	.....	.....	.....	.....	.....	.....	.....
Av. growth rate per day (mm.).....							1.34	.....						1.55

* Plotted in Fig. 2.

TABLE 9

MEASUREMENT (IN MM.) OF FIRST-GENERATION FEATHERS DERIVED FROM GRAFTING VENTRAL HALVES OF BREAST PAPILLAE VENTRALLY TO WHOLE PAPILLAE OF THE BREAST (EXPT. 931)

Days of Regeneration	Experimental											Controls		
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	Av.*	No. 1	No. 2	Av.*
24.....	15.0	23.5	20.5	8.0	17.0	11.5	18.5	13.0	7.0	15.0	15.0	26.0	23.0	24.5
28.....	24.5	34.5	29.5	16.5	22.0	19.0	28.0	19.5	12.5	24.0	23.0	32.0	31.0	31.5
32.....	32.5	43.5	38.0	24.0	28.0	26.5	33.5	25.0	18.0	32.0	30.5	38.0	40.0	39.0
36.....	41.5	53.5	46.0	32.0	36.0	36.5	44.5	32.0	26.0	42.0	39.0	51.5	49.5	50.5
40.....	50.0	65.0	55.5	41.0	43.5	46.0	54.0	37.5	34.5	52.0	48.0	60.0	55.0	57.5
44.....	58.0	71.5	62.5	50.5	50.0	54.5	62.0	49.5	41.5	61.0	56.0	67.0	61.0	64.0
48.....	65.5	80.0	71.5	59.0	61.0	6[illegible].0	71.5	60.5	52.0	70.0	66.0	77.0	72.0	74.5
52.....	74.0	87.5	78.5	68.0	68.5	74.5	80.0	70.0	60.0	80.0	74.0	85.0	83.0	84.0
56.....	83.5	96.0	86.5	78.0	76.0	84.0	88.5	80.0	70.0	88.5	83.0	92.5	88.5	90.5
60.....	91.0	99.5	94.0	87.0	83.5	91.0	93.5	88.0	78.0	97.0	90.0	98.0	101.0	99.5
64.....	98.5	103.5	101.0	97.5	90.0	99.5	100.0	97.0	87.0	106.0	98.0	103.0	106.0	104.5
68.....	101.0	107.0	105.0	106.0	97.0	106.5	107.0	107.0	99.5	114.0	105.0	105.0	107.0	106.0
72.....	104.0	107.5	108.5	113.0	101.0	115.5	109.0	117.0	109.0	117.0	110.0	.....	.....	.....
76.....	104.0	108.0	110.0	115.0	101.0	119.0	110.0	122.0	114.0	120.0	112.0	.....	.....	.....
Av. growth rate per day (mm.).....											1.47	.....		1.56

* Plotted in Fig. 3.

up to the sixtieth day for both. Their total increment in this 36-day period (between days 24 and 60) is exactly 75.0 mm. in both, thus an average growth rate of 2.10 mm. per day—a normal B rate. The situation is quite different for the heterologous group. Here, a glance at Figure 2 reveals that curve *A* (controls) is the steeper of the two. Its straight portion from days 24–52 gives an increment of 63.0 mm., or a rate of 2.25 mm. per day, whereas the chimaeric feathers represented by curve *B* extend their straight portion of the curve up to the sixty-eighth day, with a total increment of 82.0 mm., or an average of 1.86 mm. per day in the 44-day period of maximal growth. This is a maximal growth rate intermediate between the maximal growth rates of B and S. A difference in the order of 21 per cent is thus demonstrated between the control and experimental feathers of the heterologous group, as against no difference whatever (as far as growth rate is concerned) existing between the experimental and control feathers of the homologous group.

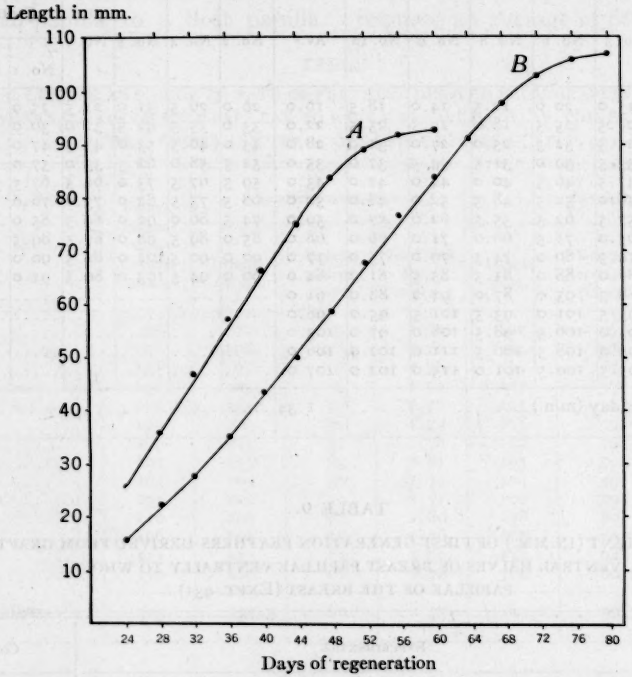

FIG. 2.—Growth curves, based on Table 8, representing (*A*) the average of 2 controls (normal breast) and 4 experimental feathers, giving no evidence of incorporation of the graft, and (*B*) average of 6 chimaeric feathers resulting from whole breast papillae after having incorporated a ventral saddle half-papilla.

One minor, but nevertheless salient, point deserves mention in passing. There was a characteristic delay in emergence from follicles which is evidenced by an initial difference in length (about 10 mm.) between the control and experimental feathers, as indicated by the curves in Figures 1, 2, and 3.

The results of these two experiments

completely rule out any possibility that the alteration in growth rate so consistently demonstrated in the chimaeric feathers can be due to the operation itself; for here the observed differences in growth rate of the homologous and heterologous feathers may be attributed to nothing other than the absence of S epidermal cells in the former and their presence in the latter.

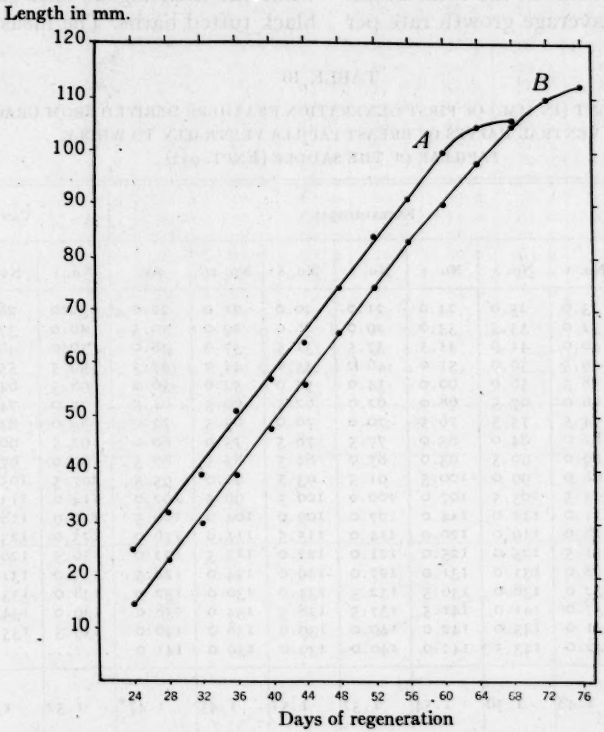

FIG. 3.—Growth curves, based on Table 9, representing (*A*) the average of 2 controls (normal breast) and (*B*) the average of 10 feathers resulting from whole breast papillae after having incorporated a ventral breast half-papilla.

This series of experments is, indeed, incomplete without grafting whole B ventral half-papillae to whole S papillae as a final control. This is a repetition of our previous type 15*a* (Lillie and Wang, 1944; see also Table 4, present article). Ten S papillae were so operated, of which 6 (Nos. 1, 2, 4, 7, 8, and 10) gave rise to chimaeric feathers, while the remainder failed to persist (Table 10). The saddle character displayed in the former, though uniformly limited to an effect on the after-feather (greatly enlarged and fluffy), was definitely identified by tufts of black barbs, which top the after-feather. These barbs, carrying completely melaninized barbules to the tip, are evidence of participation of the graft (B) epidermis. Yet the measurement of these feathers and the 2 controls (normal follicles close by), shown in Table 10, shows no appreciable difference in growth rate or otherwise.

The only difference between them is that the experimental feathers were shorter than the controls on the first day of measurement (for the same reason as stated on p. 352), but this was compensated for by a longer regeneration period. Both are very similar in the total length attained and in average growth rate per day. We conclude, therefore, that the addition of B epidermal cells to an S papilla does not affect the normal rate of growth of the host.

Our last series of experiments involved two types of tuft feathers. They were produced by combining two ventral half-papillae, B with B and B with S, with their cut surfaces fused and healed together. In line with our experience (Lillie and Wang, 1941, p. 116), this operation yielded less than 50 per cent of the expected results. Out of 10 operations of each kind, 4 tuft feathers resulted. Of these, all of the heterologous variety possess both black and reddish-brown barbs, the latter having typical S lacy tips; while the homologous ones carry totally black tufted barbs. The measurement of these feathers is presented in Table 11, their averages being plotted in Figure 4.

TABLE 10

MEASUREMENT (IN MM.) OF FIRST-GENERATION FEATHERS DERIVED FROM GRAFTING VENTRAL HALVES OF BREAST PAPILLA VENTRALLY TO WHOLE PAPILLAE OF THE SADDLE (EXPT. 932)

Days of Regeneration	Experimental							Controls		
	No. 1	No. 2	No. 4	No. 7	No. 8	No. 10	Av.	No. 1	No. 2	Av.
24	23.0	25.0	24.0	21.0	20.0	21.0	22.0	32.0	28.5	30.0
28	32.0	33.5	33.0	29.0	28.0	29.0	30.5	40.0	37.0	38.5
32	40.0	41.0	41.5	37.5	31.5	37.0	38.0	50.0	46.0	48.0
36	49.5	50.0	51.0	46.0	45.0	44.0	47.5	59.5	55.5	57.5
40	58.5	58.0	60.0	54.0	54.0	52.0	56.0	68.5	64.5	66.5
44	66.0	67.5	68.0	62.0	62.5	60.5	64.5	75.0	74.0	74.5
48	74.5	75.5	76.5	70.0	70.0	65.5	72.0	84.0	82.5	83.0
52	82.0	84.0	85.0	77.5	78.5	75.0	80.0	92.5	90.0	91.0
56	89.0	90.5	93.0	85.0	84.5	83.5	87.5	100.0	97.5	99.0
60	98.0	99.0	100.5	91.5	93.5	90.0	95.5	107.5	105.5	106.0
64	104.5	105.5	107.0	100.0	100.5	96.5	102.0	114.0	113.0	113.5
68	111.0	112.0	114.0	107.0	109.0	104.0	109.5	119.0	118.0	118.5
72	118.0	119.0	120.0	114.0	115.5	111.0	116.0	125.0	123.5	124.0
76	121.5	125.0	125.0	121.0	122.0	117.5	122.0	130.5	128.0	129.0
80	128.0	131.0	131.0	127.0	129.0	124.0	128.5	133.0	131.5	132.0
84	132.0	136.0	136.5	132.5	134.0	130.0	132.0	138.0	133.0	135.5
88	137.0	141.0	141.5	137.5	138.5	134.0	138.0	139.0	134.0	136.5
92	141.0	143.0	142.0	140.0	139.0	138.0	140.0	139.5	135.0	137.0
96	142.0	143.5	142.0	140.0	139.0	139.0	141.0	.....	.....	.....
Av. growth rate (mm.)	1.48	1.50	1.54	1.52	1.51	1.45	1.47*	1.52	1.47	1.49*

* These will be 1.52 and 1.55 mm. per day, respectively, for the experimental and control feathers if calculated on the basis of the measurement next to the last.

Little needs to be said about the significance of these results. Consistent with all the evidence presented above, the heterologous tuft feathers again showed a much lower growth rate and a longer regeneration period than the homologous controls. The average growth rates of these feathers are, respectively, 0.78 and 0.92 mm. per day, a difference of over 15

TABLE 11

MEASUREMENT (IN MM.) OF TUFT FEATHERS REGENERATING FROM HOMOLOGOUS (B + B) AND HETEROLOGOUS (B + S) COMBINATIONS OF VENTRAL HALF-PAPILLAE (EXPTS. 933 AND 934)

Days of Regeneration	Expt. 933: B+S Ventral Halves					Expt. 934: B+B Ventral Halves				
	No. 3	No. 5	No. 8	No. 9	Av.*	No. 1	No. 2	No. 5	No. 7	Av.*
24	20.0	23.0	7.0	20.0	18.0	12.0	15.5	19.5	20.5	17.0
28	24.0	28.0	12.0	24.5	22.0	18.0	25.0	25.0	28.0	24.0
32	28.0	31.0	16.0	30.0	26.0	22.0	31.0	30.5	35.5	30.0
36	30.5	33.0	20.0	35.0	29.5	28.5	38.0	37.5	40.0	36.0
40	35.0	35.5	24.5	40.0	34.0	32.0	44.0	44.0	44.0	41.0
44	38.5	39.0	28.5	43.0	37.0	37.0	51.5	51.5	50.0	47.5
48	43.0	43.0	32.0	46.0	41.0	41.5	57.0	57.0	55.0	52.5
52	47.0	46.5	36.5	48.0	44.5	47.5	62.5	59.5	57.5	57.0
56	50.5	51.0	40.5	51.0	48.0	53.0	67.5	61.0	62.5	61.0
60	56.0	55.0	44.0	55.0	52.5	59.0	72.0	63.0	68.0	65.5
64	60.0	58.0	48.0	58.0	56.0	65.0	76.5	65.5	73.0	70.0
68	64.5	60.5	52.0	62.0	60.0	69.5	78.5	67.5	76.5	73.0
72	69.5	64.0	55.5	65.0	63.5	75.0	81.0	69.5	80.5	76.5
76	74.0	67.0	59.0	69.0	67.0	78.0	81.5	70.5	82.0	78.0
80	79.0	69.5	62.0	72.5	71.0	80.0	83.0	71.0	84.0	79.5
84	82.5	72.0	65.0	74.5	73.5	81.5	84.0	71.0	85.5	80.5
88	85.5	75.5	68.5	76.5	76.5	82.0	85.0	71.0	87.0	81.0
92	87.0	77.0	73.0	79.0	79.0	.....	.....	.....	.....	.....
96	88.0	79.0	76.0	80.0	81.0	.....	.....	.....	.....	.....
100	89.0	81.5	77.5	81.0	82.0	.....	.....	.....	.....	.....
104	89.0	82.0	79.0	82.0	83.0	.....	.....	.....	.....	.....
108	89.5	82.5	80.0	83.0	84.0	.....	.....	.....	.....	.....
Av. growth rate per day (mm.)					0.78					0.92

* Plotted in Fig. 4.

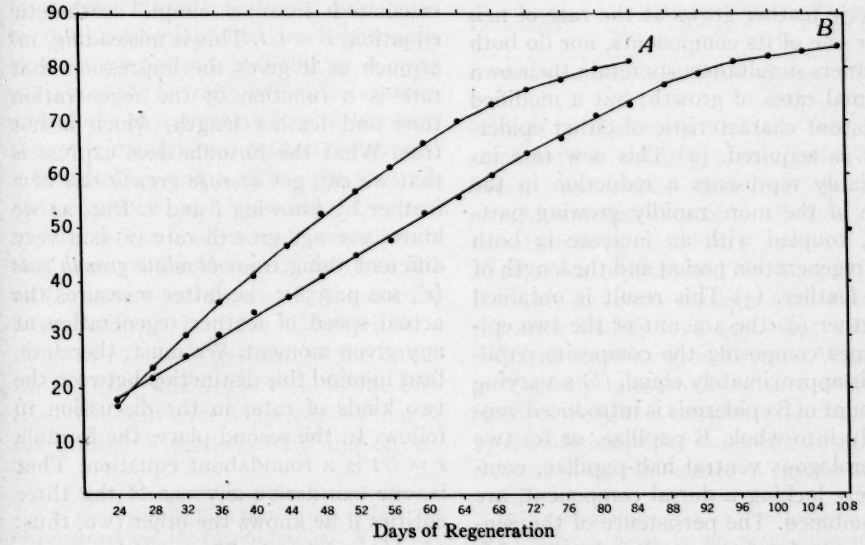

FIG. 4.—Growth curves, based on Table 11, representing (*A*) tuft (rhachis-free) feathers derived from 2 recombined breast ventral half-papillae and (*B*) chimaeric tuft feathers derived from 1 breast and 1 saddle ventral half-papillae. Both curves represent the average of 4 feathers.

per cent (see p. 360 for a discussion of the reduced rate in both these types of tuft feathers). The difference in their maximal rates is even more striking. Thus, if we take the period from days 24–64 for curve *A* and days 24–88 for curve *B* as comparable straight parts, their maximal rates are 1.32 mm. for the homologous and 0.98 mm. per day for the heterologous tuft feathers—a reduction of more than 25 per cent. These results prove that the S epidermal cells, even when incorporated within a composite papilla completely lacking a dorsal (rhachis-forming) component, are, nevertheless, capable of decreasing the growth rate of its more rapidly growing partner.

DISCUSSION

From the results considered above, it is safe to assert that the addition of S epidermis into a B papilla produces a measurable effect on the axial growth rate of the resulting feather. The analysis has shown the following: (1) A chimaeric feather grows at the rate of neither one of its components, nor do both partners simultaneously retain their own normal rates of growth; but a modified rate, not characteristic of either epidermis, is acquired. (2) This new rate invariably represents a reduction in the rate of the more rapidly growing partner, coupled with an increase in both the regeneration period and the length of the feather. (3) This result is obtained whether (*a*) the amount of the two epidermes composing the composite papillae is approximately equal, (*b*) a varying amount of S epidermis is introduced ventrally into whole B papillae, or (*c*) two heterologous ventral half-papillae, completely lacking a dorsal component, are recombined. The persistence of the general pattern of the results, despite such diversity of operative conditions, is suggestive of the qualitative, rather than the quantitative, nature of the mechanism in operation. (4) Verification of the S effect has come from the transverse chimaerae (p. 342), which identify the B, chimaeric, and S rates of growth with their respective morphologies. (5) Negative results are invariably obtained (*a*) when B papillae are grafted with homologous grafts, (*b*) when an S graft fails to become incorporated, or (*c*) when an incorporated graft is lost in a later generation (p. 347). (6) The lack of reciprocity of the observed effect is confirmed by the failure of comparable B grafts to exert an opposite effect on the growth rate of S (host) feathers (p. 354).

Since all feathers dealt with in this paper involve three measurable effects: length (l), regeneration time (t), and growth rate (r), we should devote some space in evaluating the relative role of these factors in order to arrive at an understandable interpretation of the results. In the first place, growth rate is calculated from a simple arithmetic equation, $r = l/t$. This is misleading, inasmuch as it gives the impression that rate is a function of the regeneration time and feather length, which is not true. What the formula does express is that we can get *average growth rate* of a feather by knowing l and t. But, as we know, average growth rate (r) is a very different thing from *absolute growth rate* (r', see p. 358); the latter measures the actual speed of feather regeneration at any given moment. We must, therefore, bear in mind this distinction between the two kinds of rates in the discussion to follow. In the second place, the formula $r = l/t$ is a roundabout equation. That is, one can derive any one of the three entities if he knows the other two, thus: $t = l/r$ and $l = tr$. The resulting confusion makes difficult any attempt at a per-

tinent appraisal of the three factors. It is, nevertheless, conceivable that one of the three factors probably acts in the capacity of a denominator which regulates the other two in some definite fashion, or one of them may perhaps depend on the remaining two and hence is more than a *pro forma* function of the latter. It is our immediate task to try to find a solution to this problem.

Let us begin this inquiry by finding out what sort of relationship holds between l and r. We have seen (p. 338) that the axial growth rate of typical feather-chimaerae is much closer to the S than to the B rate. This is generally consistent with all the other results (Table 12),[9] thus justifying the assumption that the magnitude of the effect is independent of the relative amount of the two kinds of epidermal cells present as well as of the manner in which they are brought together. In order to test the correlation of this reduction in rate with feather length (on the basis of the correlation indicated by normal B and S feathers, a decrease in rate is accompanied by increase in length), we present, in Table 12, a comparison of the l/r ratio (which necessarily gives days of regeneration, or t) between the experimental feathers and the normal saddle feather and between the controls of the various experiments and the normal B feather. Such a treatment reveals that none of the chimaeric feathers has an l/r ratio equal to that of the normal S feather (97.7) but that all fall within a range of 80 $\pm$ 11.3 per cent of the latter. On the other hand, the ratio of the controls turns out to be very close to that of the normal B feather (62.3 and 60, respectively).[10] This analysis seems to indicate that under normal conditions both r and l (of each tract) are constants, and hence t is also a fixed quantity; but under the experimental conditions, r and l deviate from normal values in such a way as to require a corresponding change in the value of t.

Upon a closer examination of individual cases, however, the proposed correlation cannot be substantiated, and so it must be discarded. The best evidence available for refuting a correlation between l and r has come from the two pseudo-bilateral chimaerae used as representatives (p. 343; Fig. 1, curves *B* and *E*): No. 304:9 (B/S) and No. 90:3 (S/B). Here, we have two feathers with exactly the same length (114 mm.) but widely different absolute growth rates. What is particularly noteworthy is that the two feathers required, respectively, 69 and 77 days to reach the same length; and as a result of this difference in regeneration

TABLE 12

CORRELATION BETWEEN GROWTH RATE AND LENGTH OF FEATHER AS EXPRESSED BY l/r (LENGTH TO RATE) RATIO

SOURCE OF DATA	l/r RATIO	
	Experimental Feathers	Control Feathers
Table 2	85.6	.....
Table 3	83.5	63.8
Table 5	73.0	56.0
Table 8	80.0	60.0
Table 9	76.6	68.0
Mean	80.0	62.3
Theoretical*	S=97.7	B=60.0

* Based on standard average growth rate and length (p. 337).

[9] The possible exception of data from Table 5 is due to the unusually high growth rate of both the experimental and the control feathers. A probable explanation for this is that the feathers used in this experiment belonged to the posterior breast.

[10] The tuft feathers (Table 11) are excluded from this treatment, owing to the complication arising from the absence of a rhachis-forming dorsal component in their papillae.

time, their calculated average growth rates (1.65 and 1.48 mm. per day, respectively) are different. The influence of t on these feathers is indisputable and thus refutes the idea that l and r are reciprocally modified in a chimaeric feather. To a less degree, similar indication of the important role played by t is furnished by the longitudinal (No. 301:8) and the transverse (No. 304:5) chimaerae (p. 343; Fig. 1, curves *C* and *D*). Here, the former is the longer feather but has a higher r than the latter, a fact which is again contradictory to such a correlation. We are, therefore, led to conclude that regeneration time, t, plays a prominent role in the determination of both length (l) and average growth rate (r) in the feather-chimaerae.

The foregoing discussion has served the purpose of giving the proper perspective to the role of regeneration time. At the same time, it also brings to a focus the importance of the absolute growth rate of these feathers. More explicitly, absolute growth rate is another way of expressing the instantaneous rate (r'), as represented by the formula $r' = \Delta l/\Delta t$. The important implication of such a formula seems obvious: r' is a constitutional attribute of the epidermis, which is most likely under the direct control of the process or processes of metabolism itself. However, r' has not been measured. It is related to the observed growth rate (r) that has been computed from the relation l/t. In considering underlying causal relations, it is r'—not r—that is thought to be of prime importance. To illustrate this, the B/S pseudo-bilateral chimaerae set out with, and keep, a much higher rate of regeneration than the S/B pseudo-bilateral chimaerae; this is true quite independently of the other factors. The longitudinal and transverse chimaerae cited above are also endowed with their respective different rates of growth. These differences in absolute growth rates, which in all probability are characteristic of the various types of feather-chimaerae, are well represented by the degree of steepness of the slope described by their respective curves (Fig. 1). In order to render further support to the dominating role of growth rate, we present above a schematic rearrangement of the data on the four representative feather-chimaerae (Table 3) for emphasizing the interrelationship of the three factors under discussion.

Growth rate: B/S pseudo-bilateral (1.65) > longitudinal (1.54) > transverse (1.49) > S/B pseudo-bilateral (1.48)

Regeneration time: longitudinal (91) > transverse (82) > S/B pseudo-bilateral (77) > B/S pseudo-bilateral (69)

Length: longitudinal (140) > transverse (122) > both types of pseudo-bilateral (114)

It is seen that, although there is good agreement between the order of regeneration time and feather length among the four types of chimaerae, no corresponding congruity is accorded by growth rate. Thus, the B/S pseudo-bilateral chimaera, which occupies the extreme low end in both regeneration time and length, leads all others in growth rate. The implication is clear that, of these three quantities concerned in feather regeneration, growth rate, by far, plays the most dominating role.

If a feather sets out to regenerate with a certain rate, what brings its growth to a close? Does it stop increasing in length

because it has reached a certain required length or because it has by then consumed a certain amount of time? We want to know, in other words, which is relatively the more decisive factor, *t* or *l*, now that we have reasonably placed the dominating role on rate. Some understanding of the mechanism by which a regenerating feather ceases operating is badly needed at this point. Why, for instance, does a B feather stop growing at a length of approximately 100 mm. and an S feather normally reach 130 mm. or more? Can there be a certain stimulus to which the epidermal cells react in bringing this about? If so, a possible approach to the underlying mechanism may be sought in the resorption of the pulp. Lillie (1940) found that the richly vascularized pulp in a regenerating feather is maintained at a functional level until a certain time and is then rapidly resorbed with the formation of the calamus (quill). A stimulus such as might be effected by a contraction of the subcutaneous musculature of the follicular wall (see Lillie, 1940, Fig. 18) would be sufficient to induce pulp resorption and thus bring the regeneration to a close. We may also conceive of the mechanism as occurring in another way. Since the papilla undergoes considerable shrinkage toward the end of regeneration—an act almost precisely timed with the diminishing of the pulp—some local stimulus capable of causing a contraction of the papilla may bring the same result. Such a stimulus, whatever it may be, is presumably of a localized nature (e.g., local nerve endings). In the present state of knowledge there is, however, no substantial evidence for these surmises. Even granting that such a mechanism is existent, it still remains to be explained why the mechanism normally operates for a period specific for each tract (20 or more days later in S than in B tract, for instance).

Our remaining alternative is to look to the epidermal cells for an answer. Let us reason that the epidermal cells of each tract are, by virtue of genetic constitution, capable of a limited period of activity for each regeneration. After a certain number of divisions they reach a state of fatigue and eventually cease to grow. This leads us to a concept of energy consumption.[11] The "regeneration cells" are to consume a certain amount of energy for the work of building a feather. The two entities, *work* and *energy*, are constants and, for convenience, may be further assumed to be equal in the two tracts. The B cells, however, being "faster workers" than the S cells, finish the job in less time. The deciding factor, then, evidently resides in a specific difference in the cells of the two tracts. There are two factors which account for axial increment in a growing feather: they are cell division and cell differentiation, which, according to Lillie and Juhn (1932), contribute, respectively, 10 and 90 per cent of total feather growth. Of these, cell division is, by far, the more fundamental, since upon it depends the amount of cellular material available for differentiation at any given moment. In other words, the rate of cell division determines the amount of growth of the whole feather at any time (see experimental evidence of equal axial growth rate in all parts of a feather cylinder, in Lillie, 1940, p. 151). Thus, in the ultimate analysis, the rate of cell division in the collar of a regenerating feather determines, or at least directly controls, the

[11] This concept is, by no means, new (Rubner, 1909, p. 625, Heilbrunn, 1943; MacArthur and Baillie, 1929); however, the application of its fundamental principles to the interpretation of feather regeneration is made here for the first time.

growth rate of a feather, i.e., the rate of its elongation.

The present findings can best be understood as the result of an alteration in the rate of cell division of the epidermal cells in the collar of a composite papilla. The data at hand can be interpreted by assuming the existence of a substance (metabolic product peculiar to the saddle epidermis) transmitted from the S epidermal cells to those of the B component, thereby reducing the rate of division of the B cells and thus resulting in the observed decreased rate of elongation. Such a substance, whatever it may be, should be capable of rapid diffusion throughout the collar, and a mechanism totally adequate for its operation has already been ably elaborated by Nickerson (1944) in his analysis of the barred pigmentation pattern in feathers. It is to be noted, however, that the present interpretation is tentative only, pending further inquiry. This suggestion provides an explanation for the observation that the transverse chimaerae display three distinct growth rates (p. 342).

The original idea of the rhachis acting as a "pace-setter" (Lillie and Wang, 1943, p. 18) essentially still holds. In order to understand this, it is necessary to recognize the distinction that exists between the effect of the papilla-inductor on growth rate and the effect of the S epidermis in general. The former effect works by virtue of a certain stimulating influence either directly through the action of the inductor or indirectly through the rhachis. It is universally present in all feather papillae, and its effect can readily be demonstrated in feathers whose papillae have been deprived of the inductor by operations. The extent of the resulting rate reduction in the morphologically defective feathers is in proportion to (*a*) the amount of the inductor removed (Lillie and Wang, 1944, pp. 20–21) and (*b*) the specific growth tempo characteristic of the feather tracts. The present data have shown that such an influence of the inductor may be superimposed upon that of the retarding effect of the S epidermis in the chimaeric feathers. Thus we have seen in the typical feather-chimaerae (p. 340) that, while the mean rate of the chimaerae of S/B derivation falls below that of the entire group, the mean rate of the B/S cases is above it to a comparable degree. Similarly, the fact that the homologous tuft feathers grow faster than the heterologous tuft feathers (p. 354) can be explained on the ground that, while the former reflect only the absence of the inductor, the latter are subject also to the depressing effect of the S epidermis.

The fact that B grafts in S papillae failed to bring about a detectable effect on the host growth rate (p. 353) strongly indicates the unilateral nature of the effect exerted by the S epidermis upon that of B. Nevertheless, an explanation is required as to why the host (S) rate, in this case, prevailed completely, causing the graft epidermis to go to the limit in rate reduction. Only tentative suggestions can be offered. We may stress the importance of a possible quantitative competition between the B and S epidermal cells: whereas in all S-to-B combinations the largest proportion of S epidermis was never more than 50 per cent (i.e., in the typical feather-chimaerae), it did exceed the B cells by 2:1 in the present case. When the S epidermis attains such a numerical superiority, there is reason to believe that the amount of the postulated S substance could reach a concentration sufficient to suppress completely the B rate. It is also believed that the ad-

vantage gained by the S epidermis as the host may have favored its exercising such a dominance.

We now pause for a comparison of the present results with other works dealing with rate modification in feathers.[12] A brief survey of the related literature suggests a classification based on the nature of the follicular response as follows:

1. The general stimulating effect of thyroxine, e.g., increased growth rate in replacing feathers after thyroid feeding (Cole and Reid, 1924), or, conversely, the drastic reduction following thyroidectomy (Blivaiss and Domm, 1944; cf. also Greenwood and Blyth, 1929), can readily be ascribed to changes in the basic metabolism. According to the preliminary report of Blivaiss and Domm, the rate of growth falls off 74 per cent for the B, 55 per cent for the back, and 45 per cent for the S feathers—roughly proportional to the normal growth rate of the respective tracts, the slowest growing being affected the least, and vice versa. Similarly, the uneven diurnal-growth curve reported by Lillie and Wang (1940) is interpreted on the basis of a probable correlation with the known diurnal fluctuations of basic metabolism (Barott *et al.*, 1938; Bacq, 1929; Simpson and Galbraith, 1905). Biological phenomena of this sort may find their basic interpretation in Child's (1942) widely applied and accepted theory of differential susceptibility (cf. Lillie, 1932*b*, p. 177).

2. The action of female hormone (less specifically also of thyroxine) in causing or inducing plumage feminization (Juhn, Faulkner, and Gustavson, 1931; Lillie and Juhn, 1932; Lillie, 1932*a*; Torry and Horning, 1922, 1925; Cole and Reid, 1924) belongs to a different category, so far as its effect on growth rate of the feathers is concerned. This is because the hormone-induced female rate (which was overshadowed by the far more phenomenal changes in pigmentation and structure and was hence overlooked) involves a clearly differential response from the different feather tracts. Thus, while the fast-growing B feathers are slowed down, the male (castrate) back and S feathers, which normally grow more slowly than those of the female, are concurrently considerably accelerated (Juhn, Faulkner, and Gustavson, 1931, p. 79). The female hormone presumably acts directly on the epidermis (cf. Hamilton, 1941; Willier, 1942) in bringing about this differential response; such a response is hence qualitative in nature, in this case closely associated with sex differentiation.

One thing common to the foregoing two categories of reaction is the fact that the response is induced by specific extrinsic factors in the physical environment of the follicular cells. In contrast to this are the following examples of follicular responses that result from the action of intrinsic factors.

3. The effect of the inductor, residing in the dorsal half of the dermal papilla, on growth rate is a case in point. Lillie and Wang (1944, pp. 20–21) estimated that the feathers with only half a rhachis grow at 77 per cent of the normal rate, and tuft feathers, which are completely deprived of the organizing influence of the inductor, at only 56–60 per cent. This finding (confirmed, p. 356) designates the papilla-inductor as a rate regulator in addition to its other important morphogenetic functions (Wang, 1943; also 1944*a*, p. 116). Whether this is accomplished through transmissible morphogenetic stimuli maintained throughout the regeneration or indirectly through

[12] Consult Danforth (1928, 1929) and Warren (1925) for a genetic treatment of feather growth rate.

the rhachis of the feather, as suggested by Lillie and Wang (1943), the significance lies chiefly in the inevitable quantitative relationship between the inductor and the growth rate of the feather demonstrated. The fundamental nature of this response is further borne out by our observation that, when more than one agent is at work, the respective effects on the resultant growth rate can be identified through analysis (p. 360).

4. The results of the present experiments throw further light on the intrinsic nature of rate regulation in another way. Here we put two epidermes with unlike growth rates in mutual contact in an otherwise entirely normal environment. Under these conditions we have seen a preponderantly unilateral action of the S epidermal cells on those of the B in determining the growth rate of the composite feather. The implication is that the S epidermis is unique in that its intrinsically low growth rate is somehow transmissible to the intrinsically fast-growing B cells. The interpretation advanced on page 359, if correct, serves as a preliminary approach to a better understanding of the physical basis for tract-specific differences in feather growth rates. It is presumably chemical in nature, most likely involving enzymes or catalyzers as regulators of the rate of cell division.

Due emphasis should be put on the qualitative nature of the unique property of the S epidermis. The suggested (p. 360) hypothetical substance capable of lowering growth rate may be related to the yet unsolved riddle of pigment dimorphism in the Brown Leghorn fowl (cf. Lillie and Wang, 1943, p. 18; Wang, 1943, p. 344; Willier, 1942). But one thing seems sufficiently certain: a mere quantitative correlation between growth rate and the kind of pigment to be synthesized in a feather, such as advocated by Faulkner (1932), has been disproved by the present results, since the tuft feathers (p. 353), despite their extremely low growth rate (lower than the level set by Faulkner to be the minimum for synthesis of black melanin), still retain the specific B and S pigmentation. A much more subtle mechanism than that implied by growth rate alone is, therefore, to be sought (cf. the role of growth rate in barred pattern formation, Willier and Rawles, 1944).

Another implication from these results bears on the well-established correlation between growth rate and hormone threshold (Lillie and Juhn, 1932; Juhn, Faulkner, and Gustavson, 1931; Lillie, 1932*a*, 1932*b*, and 1942; Montalenti, 1934). The feather-chimaerae, on account of their modified growth rate, present a hitherto unencountered situation in which to test the action of female hormone. Since neither the B nor the S epidermis contributing to these feathers grows at its own normal rate, it will be of interest to see whether the mutually acquired (chimaeric) growth rate resulting from the combination would, in any way, affect the response of these cells to the hormone. We hope to deal with this problem elsewhere.

SUMMARY AND CONCLUSION

The growth rate of over 300 chimaeric feathers from breast and saddle composite papillae, experimentally produced in Brown Leghorn capons by different methods of grafting, is analyzed with reference to the normal rates of the components. These methods include combinations of heterologous sagittal and frontal halves, reciprocal addition of papilla sectors, grafting of intact or hashed saddle papillae to breast, and combinations of ventral halves of breast and

saddle papillae. Representative feathers were measured throughout their regeneration with unoperated follicles as controls. Special significance has been attached to the transverse chimaerae, for they strongly suggest that, while the middle transitional (chimaeric) zone grows at a reduced rate, the saddle apex and breast base manifest, unmodified, their respective normal rates of growth. In general, comparisons between computed growth rate and length of the experimental feathers with those of the normal ones showed consistently that, whenever morphological features indicated a successful incorporation of a saddle graft in a breast papilla, the growth rate of the feather was reduced, with a concurrent increase in both the regeneration time and the total feather length attained. No such effect was observed in cases in which similar grafts failed to survive or persist, or when breast papillae received homologous grafts; nor did comparable breast grafts to saddle papillae reveal any trace of a reciprocal effect. These results are, therefore, considered as highly suggestive of a unilateral action of the saddle epidermis on that of the breast. It is tentatively suggested that the donor saddle epidermal cells produce, in the collar of the composite papilla, some substance capable of depressing the rate of cell division in the adjoining breast cells; accordingly, a diffusion mechanism is supposed to be responsible for making possible the required physical contact. Such a theoretical interpretation may lead to an understanding of the nature of a physical basis for the differential feather growth rates normal to the Brown Leghorn male and castrate.

Growth rate is also under the control of the dorsal (rhachis-forming) component in a composite papilla. Its action is independent of that of the saddle epidermis, but the two could work in conjunction with each other without, however, losing their respective identities when the combined effects on growth rate are analyzed.

A partly theoretical inquiry into the intrinsic interrelationship between growth rate, regeneration time, and length of feathers strongly indicates that growth rate plays the dominating role; and of the remaining two factors, regeneration time is of greater importance than feather length in their reciprocal relations.

The unique nature of the saddle epidermis of Brown Leghorn capons is brought out by both the present results and those already in the literature. Some general implications of the present findings are discussed.

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MATING BEHAVIOR AND THE SOCIAL HIERARCHY IN SMALL FLOCKS OF WHITE LEGHORNS[1]

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OUR original interest in this study stems from the reports on social organization in the flocks of common domestic fowl by Schjelderup-Ebbe (1922, 1935). The avian hierarchy in flocks of hens and of other birds has received much analytical attention by Allee and his associates (see Literature Cited). These studies have been concerned with endocrine, physiological, and psychological aspects of group behavior and have recently been summarized by Allee (1942, 1943, 1945) and by Collias (1944). It early became apparent that certain differences were associated with position in the so-called "peck-order" of the flock. High-ranking hens possess a greater freedom of activity as compared with those of low social status; for example, they have precedence to food (Masure and Allee, 1934). Many aspects of observed relations to social status are summed up in the statement that fewer eggs are laid by hens in the lower half of the social order than by those composing the more aggressive half (Sanctuary, 1932). We have here attempted to determine a part of the relation of the social order to the mating behavior in such flocks. In many ways the present study is a continuation and extension of the work of Skard (1937). A preliminary abstract of some of the results has been published by one of us (Guhl, 1941).

[1] We are indebted to Professor L. F. Payne and Dr. D. C. Warren for counsel given to the first author concerning the interpretation of the data, to Professor Sewall Wright for advice concerning statistical methods, and to Mr. Peter Frank for extensive statistical calculations.

[2] A. M. Guhl is now located at Kansas State College, and N. E. Collias has entered the United States Army.

ANIMALS AND THEIR TREATMENT

All animals used in these studies were White Leghorns; we have reason to believe that all were pure-bred representatives of that breed. Observations by the first author on two flocks of cocks and hens provided the main body of data to be analyzed here; data have also been considered that were obtained during previous observations on some of these birds, as well as on others. These flocks were housed in the poultry-house at Whitman Laboratory in pens 14.5 feet long, 4.5–5.0 feet wide, and 8 feet high. They were continuously illuminated (between 6:00 A.M. and 6:00 P.M.) by a 60-watt ceiling lamp near the center of each pen in addition to the natural light entering from south windows. During the cold months the pens were heated by steam radiators and were roughly controlled at a temperature of 10°–15° C. A more complete description of the general situation may be found elsewhere (Allee, Collias, and Lutherman, 1939). Mash and water were available at all times, and a mixture of grain was fed during the afternoons. Feeding and general care, including cleaning of the pens, were in the hands of the observer, to whom the birds became accustomed and whom they appeared to ignore during observations as he watched them from the outside of the pen.

Flock D.—This flock contained 7 hens,

which were mature when obtained in October, 1938. The 4 cocks were received as newly hatched chicks in August, 1939. They were reared with females of their own age and were separated from them about 3 weeks after treading (copulation) had become frequent. These males were then kept in a pen by themselves from early January, 1940, to April and had not been exposed to this flock of hens before the present experiments. Straight-line peck-orders prevailed among both hens and cocks; a change in order occurred among the cocks during the course of the observations and will be noted later.

Beginning April 13, 1940, the males were placed singly and successively into the pen of the hens of flock D on alternate days. This procedure, which left the hens without males every other day, was taken as a precaution against a probable carry-over of certain reactions of the hens toward a given cock that might influence their behavior toward his successor. The sequence in which the males were introduced remained the same. Each cock was placed in the pen during the preceding night or early morning of its scheduled day of exposure to the hens. The recorded observations of 2 hours' duration (1 hour on the initial day) were made between 3:00 and 6:00 P.M. until each male was exposed ten times and for a total of 19 hours of observation.

On June 22, all 4 cocks were introduced together into the pen, where they remained with the hens. No records were made until July 4, to allow for adjustment. The initially intense interaction caused the hens to remain on the roost. Cock IV died on July 24; and on September 8 the dominant cock, *I*, was removed for a period of 30 days, for reasons to be discussed later; cocks II and III were left with the hens throughout this period of observation.

Flock F.—These 9 hens and 4 cocks were received in February, 1941, when nearly 1 year old. All of the birds but cock VIII came from the same flock. Pecking triangles occurred among the hens for 10 days; hen GB developed a paralysis and was removed on May 27; and on the same day YG gained dominance over PB and so initiated the only triangle that persisted through most of the experiment with this flock.

The males of this group, especially VI, were not socially aggressive and did relatively little pecking. Threats and avoiding reactions were used to determine their peck-order; back-pecks and brief fights, more threatening than final, were common throughout much of the experiment. However, the order given in Tables 1–4 was definite for the period covering the time during which treading data were taken. When all the males were placed in the pen of these hens, pecking by the males occurred more frequently and the same order prevailed. Cock *I* of flock D was included in part of the observations of flock F for general comparative purposes.

Each day for a total of 60 days, beginning April 26, 1941 (exclusive of a 5-day interval at the end of the fourth round for the males), one cock in a wire cage was placed within the pen of the hens. He was released during the observation period of 30 minutes and was then replaced by another caged cock in preparation for the observations of the following day. This method greatly increased the number of matings and the amount of other intersexual behavior that was exhibited during the observation period (cf. Skard, 1937). All 4 cocks of flock F were introduced into the pen for permanent residence on July 3 and observed for 60 minutes daily for 48 days.

Flock G.—This group of 6 hens was

used as an accessory flock in certain tests of the males of flock D. It was composed of hens from several sources, of various ages, and also included a poulard.

Preliminary observations were made using the following additional flocks:

Flock A6.—The 6 birds composing this flock had lived together for over 4 months when observations started early in February, 1939. The flock included 4 moderately inbred young White Leghorn hens, received as pullets from Iowa State College, and 2 old hens from flock D.

Flock E.—Flock E was a small lot of hens from an Indiana Farm Bureau strain that had been intraflock bred for 6 years. These yearling hens were culled from a large flock and were received by us late in April, 1939; observations on mating frequency were begun after the hens had been under our care for a few weeks.

Two cocks, other than those mentioned for flocks D and F, were used in certain earlier tests. These were obtained from a local poultry farm when about 7 months old. One of these was used with flocks A6 and E; the other was with the flock-D hens in 1939 when the data for these three flocks (Table 9) were obtained. Both these males were very active sexually.

To recapitulate: The cocks and hens were kept in unisexual flocks to determine their respective peck-orders. The males were then placed singly with the hens of their respective flocks every other day in flock D and daily in flock F to study the sexual behavior of the hens in association with each individual cock. The order of introduction of the cocks into the pen of the hens on successive days remained unchanged. After several weeks of such observations the cocks of each flock were placed simultaneously into the pen of their respective female flockmates, where they remained for another series of observations on sexual behavior under these more complicated conditions. At certain times the same birds were used to study social discrimination, the results of which have been reported separately (Guhl, 1942). These flocks were under observation during the latter part of the afternoon, when matings occur most frequently (Heuser, 1916; Philips, 1919; Upp, 1928; Skard, 1937; Parker, McKenzie, and Kempster, 1940). The peck-orders given in Tables 1 and 2 were based on more than 9,000 pecks or threats; nearly 1,000 matings were observed during this particular series of observations and experiments.

OBSERVATIONS

Tables 1 and 2 present many of the basic data obtained in flock D and flock F in 1940–41. Table 1 summarizes the data obtained when the cocks were introduced singly and successively into the pens of their respective flocks of hens. Table 2 gives a similar summary for the observations when all 4 cocks of each flock were continuously in the pens of the hens. The results for each flock are separated in the body of the tables. The hens are designated by letters and are listed according to their position in the peck-order, beginning with the alpha hen. The cocks are indicated by Roman numerals, and their peck-order reads from left to right. It will be noted that the male order shown in Table 2 differs from that in Table 1, since in the meantime cock IV had defeated cock III.

The column headings marked "Treadings," "Courtings," "Sex Invitations," and "Avoidances" (described below) indicate the types of behavior tabulated; no avoidances were recorded in Table 1 for flock D, as the value of such data was not appreciated when this study began.

Early in the observations of flock D it was noted that hens would fly to the roost or the top of the nest boxes, where they remained for varying lengths of the time and where they were usually out of contact with the cocks. No matings occurred on these perches; and, since sustained avoidance would become a major factor in computing the frequency of sexual behavior between hens and cocks, it was found necessary to record, in minutes, the time each hen and each cock spent on the floor, where it was exposed on the floor, where it was exposed to the opposite sex. From such data each bird has been ranked, within its flock and sex, according to its mean rate in mating, in courting, and in giving or receiving "sex

TABLE 1

HETEROSEXUAL BEHAVIOR WHEN COCKS WERE INTRODUCED SINGLY AND SUCCESSIVELY INTO THE PEN OF THE HENS

Results are given by individuals and by totals for each hen and each cock. (See Table 3)

Peck-Order	♂ *I*				♂ II				♂ III				♂ IV								Totals			
	Treadings	Courtings	"Sex Invitations"	Avoidances	Treadings	Courtings	"Sex Invitations"	Avoidances	Treadings	Courtings	"Sex Invitations"	Avoidances	Treadings	Courtings	"Sex Invitations"	Avoidances	Treadings	Courtings	"Sex Invitations"	Avoidances	Treadings	Courtings	"Sex Invitations"	Avoidances
	Flock D (40 Observations between April 13 and June 20, 1940)																							
♀♀																								
GY....	2	10	0	...	6	10	0	...	6	10	0	...	1	4	0	...	...	...	...	...	15	34	0	...
BB....	2	20	0	...	7	9	0	...	5	9	0	...	0	1	0	...	...	...	...	...	14	39	0	...
GG....	6	16	0	...	2	14	0	...	4	11	0	...	3	11	0	...	...	...	...	...	15	52	0	...
BR....	10	11	1	...	13	8	0	...	13	26	0	...	8	17	3	...	...	...	...	...	44	62	4	...
RY....	9	15	5	...	6	10	0	...	6	24	2	...	8	20	4	...	...	...	...	...	29	69	11	...
YY....	11	24	6	...	7	23	0	...	7	31	2	...	13	37	1	...	...	...	...	...	38	115	9	...
RW...	5	25	1	...	6	10	0	...	6	13	0	...	5	21	0	...	...	...	...	...	22	69	1	...
Totals	45	121	13	...	47	84	0	...	47	124	4	...	38	111	8	...	...	...	...	...	177	440	25	...
	Flock F (60 Observations between April 26 and June 30, 1941)																							
♀♀	♂ *I**				♂ V				♂ VI				♂ VII				♂ VIII				Totals			
WG...	4	29	3	22	9	20	11	12	1	56	3	47	4	21	3	16	5	27	3	36	23	153	23	132
RG....	8	25	6	18	5	7	7	6	3	17	9	9	2	3	2	6	8	24	6	32	26	76	30	71
PB....	5	22	2	18	8	18	3	15	7	32	6	27	6	16	12	16	5	23	2	35	31	111	25	111
YB....	1	32	0	31	2	14	4	11	3	22	6	16	7	24	6	9	2	22	6	21	15	114	22	88
RB....	0	14	0	20	1	15	2	26	1	29	0	30	2	12	2	13	3	18	3	35	7	88	7	124
YG†...	9	46	2	30	6	18	12	12	14	34	5	18	10	15	12	6	5	30	5	34	44	143	36	100
GB‡...	2	9	0	9	2	11	1	16	0	6	1	6	0	4	0	6	4	7	4	15	8	37	6	52
YR....	7	25	3	13	18	28	4	7	11	22	9	8	6	11	3	6	15	55	5	46	57	141	24	80
WR...	7	35	3	23	7	17	6	7	11	39	5	17	6	23	2	15	9	46	11	50	40	160	27	112
Totals	43	237	19	184	58	148	50	112	51	257	44	177	43	129	42	93	56	252	45	304	251	1,023	200	870

* Cock *I* was not housed with the F-cocks.

† Hen YG gained dominance over PB on May 27.

‡ Hen GB died May 30.

TABLE 2

HETEROSEXUAL BEHAVIOR WHEN ALL 4 COCKS WERE CONTINUOUSLY IN THE PEN OF THE HENS

Results are given by individuals and by totals for each hen and each cock. (See Table 4)

Peck-Order	♂ *I* Treadings	♂ *I* Courtings	♂ *I* "Sex Invitations"	♂ *I* Avoidances	♂ II Treadings	♂ II Courtings	♂ II "Sex Invitations"	♂ II Avoidances	♂ IV Treadings	♂ IV Courtings	♂ IV "Sex Invitations"	♂ IV Avoidances	♂ III Treadings	♂ III Courtings	♂ III "Sex Invitations"	♂ III Avoidances	Totals Treadings	Totals Courtings	Totals "Sex Invitations"	Totals Avoidances
Flock D: Period A (29 Observations between July 4 and August 4, 1940)																				
♀ ♀	♂ *I*				♂ II				♂ IV				♂ III				Totals			
GY	7	31	2	28	2	3	0	19	0	0	0	0	0	0	0	6	9	34	2	53
BB	5	44	0	38	2	15	0	49	1	8	0	7	0	1	0	8	8	68	0	102
GG	9	27	0	23	0	10	0	27	4	14	0	11	0	1	0	1	13	52	0	62
BR	17	52	2	32	4	16	0	31	11	34	0	26	0	0	0	6	32	102	2	95
RY	22	23	10	19	3	11	0	43	4	11	0	28	0	0	0	5	29	45	10	95
YY	26	55	7	33	1	13	0	25	12	12	0	10	0	1	0	2	39	81	7	70
RW	2	10	0	9	1	1	0	7	1	4	0	7	0	0	0	0	4	15	0	23
Totals	88	242	21	182	13	69	0	201	33	83	0	89	0	3	0	28	134	397	21	500
Flock D: Period B (34 Observations between August 5 and September 8, 1940)																				
Totals	69	198	28	174	1	45	0	258	Died				0	0	0	48	70	243	28	480
Flock D: Period C (31 Observations between September 9 and October 11, 1940)																				
Totals	58*	125*	22*	181*	1	17	0	89	Died				4	5	2	116	5	22	2	205
Flock F (48 Observations between July 3 and August 20, 1941)																				
♀ ♀	♂ V				♂ VI				♂ VII				♂VIII				Totals			
WG	16	84	4	59	2	23	1	18	1	32	0	25	17	97	0	159	36	236	5	261
RG†	5	41	0	27	10	47	11	31	0	8	0	15	6	38	0	65	21	134	11	138
PB	16	96	0	72	8	76	7	58	2	25	0	26	10	66	1	133	36	263	8	289
YB	0	29	0	26	7	47	5	29	1	26	1	28	0	16	0	55	8	118	6	138
RB	0	14	0	13	3	23	1	22	0	5	0	11	0	26	0	60	3	68	1	106
YG‡	8	78	2	54	6	51	3	38	2	15	1	17	6	63	0	139	22	207	6	248
YR	21	111	1	79	10	76	3	56	0	14	1	14	11	79	1	119	42	280	6	268
WR	7	61	0	38	20	94	7	69	1	19	1	18	2	71	0	107	30	245	8	232
Totals	73	514	7	368	66	437	38	321	7	144	4	154	52	456	2	837	198	1,551	51	1,680

* Cock *I* with flock G.

† Hen RG died August 2.

‡ Hen YG pecked PB.

invitations" and to the mean rate at which the hens avoided the cocks. These rates and ranks are given in Tables 3 and 4, in terms of (*a*) the frequency of each type of behavior as the mean number per hour and (*b*) the relative rank for each bird among the members of its flock and sex in its participation in these types of behavior. Equal ranking is indicated by half-steps. The same method of ranking was used with flock F, although the number of minutes each bird spent on the floor was the same for all individuals, since the roost and nest boxes were screened off during observation time. Mean rates are also given for each hen and each cock and for the sexes as groups. The latter rates were computed from total observations (Tables 1 and 2) and aggregate time rather than as the means of the rates given in the body of these tables.

OBSERVATIONS ON GENERAL MATING BEHAVIOR WITHIN FLOCKS

The initial sexual reactions of the cocks toward a hen were recorded as courtings and involved such behavior as raising the hackle while pointing the head toward a female; catching a hen by the comb or neck; placing a foot on the saddle of a prospective mate; or pursuing her on the run. The wing-flutter or so-called "waltz," also often included as a form of courting, was recorded separately in the protocol, since this behavior seems to be characteristically somewhat different from other courtship patterns. In the waltz the cock lowers and flutters the wing on the opposite side from the female and makes an arc about the hen with a series of short, quick steps.

Often the hens seem to be sexually indifferent to the presence of the cock, but they may squat or crouch before him; such behavior by the hen was recorded as a "sex invitation." A hen may respond to active courtship by squatting, in which case copulation usually follows; she may be indifferent; or she may avoid the male. Her avoidance may take various forms with varying intensities, from merely stepping aside to running; or if the male mounts her, she may struggle to get away; or she may go to the roost and remain there, thus giving a sustained avoidance.

The movement of a hen was not considered as avoidance unless some overt behavior by the cock toward her could be associated with her reaction. Most of the recorded avoidances were responses to courting or to attempts to tread. No records of avoidance were taken when the flock-D males were introduced singly into the pen of the D-hens. Avoiding reactions may be accompanied by vocalizations ranging from a faint cry to a loud squawk. The types of sexual behavior we have observed are similar to those outlined by Skard (1937).

It is well known that to copulate the male mounts the squatting hen, grasps her comb or hackle with his beak, and balances himself on her back with a treading motion. The hen crouches low and moves her tail to one side, while the cock rears up and, spreading his tail, the vents meet. An attempt was made to determine and record whether the copulations were initiated by the male or by the female, or simultaneously by both.

In some instances the cock mounted a struggling hen and such matings appeared to be forced. As compared with the obviously male-initiated and female-initiated ("invited") matings, there is a third category, composed of incidences in which mounting by the cock and crouching by the hen occurred simultaneously. In the latter the initiator could not be determined, since the crouch may have

TABLE 3

HETEROSEXUAL BEHAVIOR PATTERNS WHEN THE COCKS WERE INTRODUCED SINGLY AND SUCCESSIVELY INTO THE PEN OF THE HENS*

(See Table 1 and p. 370)

Peck-Order	Treadings		Courtings		"Sex Invitations"		Avoidances		Treadings		Courtings		"Sex Invitations"		Avoidances		Treadings		Courtings		"Sex Invitations"		Avoidances		Treadings		Courtings		"Sex Invitations"		Avoidances		Treadings		Courti	
	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	
	Flock D (40 Observations between April 13 and June 20, 1940)																																	
♀♀	♂ *I*								♂ II								♂ III								♂ IV									
GY	0.17	7	0.89	6.5	0.00	6	.....	.....	0.91	6	1.53	5	0.00	....	.....	.....	0.57	4.5	0.95	7	0.00	....	.....	.....	0.09	6	0.37	6	0.00	5.5	.....	.....	.....	.....	.....
BB	0.21	6	2.10	1.0	0.00	6	.....	.....	1.16	4	1.50	6	0.00	....	.....	.....	0.61	2.0	1.09	6	0.00	....	.....	.....	0.00	7	0.10	7	0.00	5.5	.....	.....	.....	.....	.....
GG	0.62	4	1.68	4.0	0.00	6	.....	.....	0.34	7	2.41	3	0.00	....	.....	.....	0.48	7.0	1.32	4	0.00	....	.....	.....	0.31	5	1.15	5	0.00	5.5	.....	.....	.....	.....	.....
BR	0.81	1	0.89	6.5	0.08	3	.....	.....	2.20	2	1.36	7	0.00	....	.....	.....	1.12	1.0	2.24	3	0.00	....	.....	.....	0.65	3	1.38	4	0.24	2.0	.....	.....	.....	.....	.....
RY	0.72	3	1.20	5.0	0.40	2	.....	.....	1.25	3	2.08	4	0.00	....	.....	.....	0.59	3.0	2.38	2	0.19	....	.....	.....	0.72	2	1.80	2	0.36	1.0	.....	.....	.....	.....	.....
YY	0.79	2	1.73	3.0	0.43	1	.....	.....	1.00	5	3.28	2	0.00	....	.....	.....	0.55	6.0	2.46	1	0.16	....	.....	.....	0.94	1	2.68	1	0.07	3.0	.....	.....	.....	.....	.....
RW	0.38	5	1.91	2.0	0.07	4	.....	.....	2.40	1	4.00	1	0.00	....	.....	.....	0.57	4.5	1.23	5	0.00	....	.....	.....	0.41	4	1.72	3	0.00	5.5	.....	.....	.....	.....	.....
Mean rates	0.54	3	1.47	3.0	0.15	1	.....	.....	1.21	1	2.17	1	0.00	4	.....	.....	0.65	2.0	1.72	2	0.05	3	.....	.....	0.47	4	1.39	4	0.10	2.0	.....	.....	.....	.....	.....
Rates of ♂♂	3.05	3	8.23	2.0	0.88	1	.....	.....	4.56	1	8.15	3	0.00	4	.....	.....	3.31	2.0	8.73	1	0.28	3	.....	.....	2.46	4	7.20	4	0.52	2.0	.....	.....	.....	.....	.....
	Flock F (60 Observations between April 26 and June 30, 1941)																																	
♀♀	♂ *I*								♂ V								♂ VI								♂ VII									
WG	0.65	7	4.75	4	0.49	3.5	3.61	4.0	1.47	2	3.28	3.0	1.80	2	1.97	4.5	0.16	7.5	9.18	1	0.49	7.0	7.70	1	0.65	6.0	3.44	3	0.49	5.0	2.62	1.5	0.82	6	4.43
RG	1.31	2	4.09	6	0.98	1.0	2.95	7.5	0.82	6	1.15	9.0	1.15	3	0.98	9.0	0.49	5.5	2.79	8	1.47	2.0	1.47	8	0.32	7.5	0.49	9	0.32	7.0	0.98	8.5	1.31	4	3.93
PB	0.82	5	3.61	7	0.32	5.5	2.95	7.5	1.31	3	2.95	4.5	0.49	7	2.46	3.0	1.15	4.0	5.24	4	0.98	3.5	4.43	3	0.98	4.5	2.62	4	1.97	1.5	2.62	1.5	0.82	6	3.77
YB	0.16	8	5.23	3	0.00	8.0	5.08	1.0	0.32	8	2.29	8.0	0.65	6	1.80	6.0	0.49	5.5	3.61	7	0.98	3.5	2.62	6	1.15	3.0	3.93	1	0.98	3.0	1.47	6.0	0.32	9	3.61
RB	0.00	9	2.29	9	0.00	8.0	3.28	6.0	0.16	9	2.46	7.0	0.32	9	4.26	2.0	0.16	7.5	4.75	5	0.00	9.0	4.91	2	0.32	7.5	1.97	7	0.32	7.0	2.13	5.0	0.49	8	2.95
YG‡	1.47	1	7.54	1	0.32	5.5	4.91	2.0	0.98	5	2.95	4.5	1.97	1	1.97	4.5	2.29	1.0	5.57	3	0.82	5.5	2.95	4	1.64	1.0	2.46	5	1.97	1.5	0.98	8.5	0.82	6	4.91
GB§	0.80	6	3.60	8	0.00	8.0	3.60	5.0	0.80	7	4.40	2.0	0.40	8	6.40	1.0	0.00	9.0	2.40	9	0.40	8.0	2.40	7	0.00	9.0	1.60	8	0.00	9.0	2.40	4.0	1.60	2	2.80
YR	1.25	3	4.46	5	0.53	2.0	2.32	9.0	3.21	1	5.00	1.0	0.71	5	1.25	7.0	1.96	2.0	3.93	6	1.61	1.0	1.43	9	1.17	2.0	2.16	6	0.58	4.0	1.17	7.0	3.00	1	11.00
WR	1.15	4	5.73	2	0.49	3.5	3.77	3.0	1.15	4	2.79	6.0	0.98	4	1.15	8.0	1.80	3.0	6.39	2	0.82	5.5	2.79	5	0.98	4.5	3.77	2	0.32	7.0	2.46	3.0	1.47	3	7.54
Mean rates	0.85	....	4.68	.....	0.37	.....	3.65	.....	1.14	1	2.92	3.0	0.98	1	2.21	3.0	1.01	3.0	5.12	1	0.87	3.0	3.50	2	0.86	4.0	2.57	4	0.85	4.0	1.85	4.0	1.11	2	5.03
Rates of ♂♂	7.05	....	38.85	.....	3.11	.....	30.16	.....	9.50	1	24.26	3.0	8.19	1	18.36	3.0	8.36	3.0	42.13	1	7.21	3.0	29.01	2	7.05	4.0	21.14	4	6.88	4.0	15.24	4.0	9.18	2	41.31

* Fr. = frequency; Rk. = rank. Italic figures indicate rank.

† Cock *I* was hot housed with the F-cocks.

‡ Hen YG gained dominance over PB on May 27.

§ Hen GB died May 30.

TABLE 3

OR PATTERNS WHEN THE COCKS WERE INTRODUCED SINGLY AND SUCCESSIVELY INTO THE PEN OF THE HENS*

(See Table 1 and p. 370)

Flock D (40 Observations between April 13 and June 20, 1940)

Treadings		Courtings		"Sex Invitations"		Avoidances		Treadings		Courtings		"Sex Invitations"		Avoidances		Treadings		Courtings		"Sex Invitations"		Avoidances		Treadings		Courtings		"Sex Invitations"		Avoidances	
Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.
								♂ III								♂ IV												Mean Rates			
0.57	4.5	0.95	7	0.00	.....	.....	....	0.09	6	0.37	6	0.00	5.5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0.38	7	0.87	7	0.00	6	.....	....
0.61	2.0	1.09	6	0.00	.....	.....	....	0.00	7	0.10	7	0.00	5.5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0.41	6	1.16	6	0.00	6	.....	....
0.48	7.0	1.32	4	0.00	.....	.....	....	0.31	5	1.15	5	0.00	5.5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0.45	5	1.56	4	0.00	6	.....	....
1.12	1.0	2.24	3	0.00	.....	.....	....	0.65	3	1.38	4	0.24	2.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1.04	1	1.47	5	0.09	3	.....	....
0.59	3.0	2.38	2	0.19	.....	.....	....	0.72	2	1.80	2	0.36	1.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0.75	3	1.78	3	0.28	1	.....	....
0.55	6.0	2.46	1	0.16	.....	.....	....	0.94	1	2.68	1	0.07	3.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0.80	2	2.43	1	0.19	2	.....	....
0.57	4.5	1.23	5	0.00	.....	.....	....	0.41	4	1.72	3	0.00	5.5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0.57	4	1.79	2	0.03	4	.....	....
0.65	2.0	1.72	2	0.05	3	.....	....	0.47	4	1.39	4	0.10	2.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0.65	....	1.61	.....	0.09	....	.....	....
3.31	2.0	8.73	1	0.28	3	.....	....	2.46	4	7.20	4	0.52	2.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3.23	....	8.02	.....	0.45	....	.....	....

Flock F (60 Observations between April 26 and June 30, 1941)

								♂ VI								♂ VII								♂ VIII								Mean Rates							
0.16	7.5	9.18	1	0.49	7.0	7.70	1	0.65	6.0	3.44	3	0.49	5.0	2.62	1.5	0.82	6	4.43	4	0.49	7.5	5.90	4.0	0.75	6	5.03	3.0	0.75	6	4.34	1
0.49	5.5	2.79	8	1.47	2.0	1.47	8	0.32	7.5	0.49	9	0.32	7.0	0.98	8.5	1.31	4	3.93	5	0.98	4.5	5.24	8.0	0.85	5	2.50	9.0	0.98	2	2.33	9
1.15	4.0	5.24	4	0.98	3.5	4.43	3	0.98	4.5	2.62	4	1.97	1.5	2.62	1.5	0.82	6	3.77	6	0.32	9.0	5.73	5.5	1.02	4	3.65	6.0	0.82	5	3.65	5
0.49	5.5	3.61	7	0.98	3.5	2.62	6	1.15	3.0	3.93	1	0.98	3.0	1.47	6.0	0.32	9	3.61	7	0.98	4.5	3.44	9.0	0.49	8	3.75	5.0	0.72	7	2.89	8
0.16	7.5	4.75	5	0.00	9.0	4.91	2	0.32	7.5	1.97	7	0.32	7.0	2.13	5.0	0.49	8	2.95	8	0.49	7.5	5.73	5.5	0.23	9	2.89	8.0	0.23	9	4.07	3
2.29	1.0	5.57	3	0.82	5.5	2.95	4	1.64	1.0	2.46	5	1.97	1.5	0.98	8.5	0.82	6	4.91	3	0.82	6.0	5.57	7.0	1.44	2	4.70	4.0	1.18	1	3.29	6
0.00	9.0	2.40	9	0.40	8.0	2.40	7	0.00	9.0	1.60	8	0.00	9.0	2.40	4.0	1.60	2	2.80	9	1.60	2.0	6.00	3.0	0.64	7	2.96	7.0	0.48	8	4.16	2
1.96	2.0	3.93	6	1.61	1.0	1.43	9	1.17	2.0	2.16	6	0.58	4.0	1.17	7.0	3.00	1	11.00	1	1.00	3.0	9.20	1.0	2.12	1	5.26	1.5	0.89	3	2.98	7
1.80	3.0	6.39	2	0.82	5.5	2.79	5	0.98	4.5	3.77	2	0.32	7.0	2.46	3.0	1.47	3	7.54	2	1.80	1.0	8.19	2.0	1.31	3	5.26	1.5	0.88	4	3.68	4
1.01	3.0	5.12	1	0.87	3.0	3.50	2	0.86	4.0	2.57	4	0.85	4.0	1.85	4.0	1.11	2	5.03	2	0.90	2.0	6.06	1.0	0.99	....	4.05	.....	0.79	....	3.45	....
8.36	3.0	42.13	1	7.21	3.0	29.01	2	7.05	4.0	21.14	4	6.88	4.0	15.24	4.0	9.18	2	41.31	2	7.37	2.0	49.83	1.0	8.26	....	33.65	.....	6.58	....	28.62	....

‡ Hen YG gained dominance over PB on May 27.

§ Hen GB died May 30.

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TABLE 4

HETEROSEXUAL BEHAVIOR PATTERNS WHEN ALL 4 COCKS WERE CONTINUOUSLY IN THE PEN OF THE HENS*

(See Table 2 and p. 370)

Peck-Order	Treadings		Courtings		"Sex Invitations"		Avoidances		Treadings		Courtings		"Sex Invitations"		Avoidances		Treadings		Courtings		"Sex Invitations"		Avoidances		Treadings		Courtings		"Sex Invitations"		Avoidances		Treadings		Courtings	
	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.
	Flock D: Period A (29 Observations between July 4 and August 4, 1940)																																	
♀♀	♂ I								♂ II								♂ IV								♂ III								Mean R	
GY.....	0.38	5	1.72	4	0.11	3	1.55	4	0.35	3.5	0.53	6	0.00	.....	3.39	6	0.00	7	0.00	7	0.00	....	0.00	7	0.00	.....	0.00	....	0.00	....	33.33	3	0.28	6	1.08	7
BB.....	0.23	7	2.07	3	0.00	6	1.78	1	0.35	3.5	2.67	2	0.00	.....	8.75	1	0.10	6	0.82	6	0.00	....	0.72	6	0.00	.....	6.25	....	0.00	....	50.00	1	0.21	7	1.84	3
GG.....	0.50	4	1.50	5	0.00	6	1.28	6	0.00	7.0	2.32	4	0.00	.....	6.27	3	0.46	3	1.60	2	0.00	....	1.26	4	0.00	.....	9.09	....	0.00	....	9.09	6	0.41	4	1.67	4
BR.....	0.88	3	2.69	2	0.10	4	1.65	3	0.78	1.0	3.13	1	0.00	.....	6.07	4	1.14	2	3.54	1	0.00	....	2.70	2	0.00	.....	0.00	....	0.00	....	37.50	2	0.93	2	2.97	1
RY.....	1.08	2	1.13	7	0.48	1	0.94	7	0.60	2.0	2.20	5	0.00	.....	8.60	2	0.45	4	1.25	4	0.00	....	3.18	1	0.00	.....	0.00	....	0.00	....	31.25	4	0.84	3	1.31	5
YY.....	1.36	1	2.87	1	0.36	2	1.72	2	0.18	6.0	2.45	3	0.00	.....	4.71	5	1.31	1	1.31	3	0.00	....	1.09	5	0.00	.....	6.25	....	0.00	....	12.50	5	1.15	1	2.40	2
RW.....	0.29	6	1.49	6	0.00	6	1.33	5	0.34	5.0	0.34	7	0.00	.....	2.41	7	0.30	5	1.21	5	0.00	....	2.12	3	0.00	.....	0.00	....	0.00	....	0.00	7	0.31	5	1.16	6
Mean rates..	0.71	1	1.97	3	0.17	1	1.48	4	0.38	3.0	2.02	2	0.00	.....	5.89	2	0.58	2	1.46	4	0.00	....	1.56	3	0.00	4	3.00	1	0.00	....	28.00	1	0.62	.....	1.85	....
Rates of ♂♂..	3.18	1	8.76	1	0.76	1	6.59	3	1.34	3.0	7.11	2	0.00	.....	20.72	2	2.53	2	6.38	3	0.00	....	6.84	4	0.00	4	2.50	4	0.00	....	23.33	1	2.54	.....	7.54	....
	Flock D: Period B (34 Observations between August 5 and September 8, 1940)																																	
Mean rates..	0.81	1	2.35	1	0.33	1	2.06	3	0.04	2	1.78	2	0.00	.....	1.20	1	Died								0.00	3	0.00	3	0.00	3	3.31	2	0.56	.....	1.96	....
Rates of ♂♂..	3.41	1	9.80	1	1.38	1	8.61	2	0.10	2	4.45	2	0.00	.....	25.54	1									0.00	3	0.00	3	0.00	3	8.57	3	1.95	.....	6.76	....
	Flock D: Period C (31 Observations between September 9 and October 11, 1940)																																	
Mean rates..	1.15	†	2.48	†	0.44	†	3.60	†	0.02	2	0.36	1	0.00	2	1.86	2	Died								0.08	1	0.10	2	0.04	1	2.44	1	0.05	.....	0.23	....
Rates of ♂♂..	6.04	.....	13.02	....	2.29	....	18.85	....	0.12	2	2.15	1	0.00	2	11.26	2									0.48	1	0.60	2	0.24	1	13.97	1	0.30	.....	1.35	....
	Flock F (48 Observations between July 3 and August 20, 1941)																																	
♀♀	♂ V								♂ VI								♂ VII								♂ VIII								Mean	
WG.....	0.35	2.5	1.84	3	0.08	....	1.29	3	0.04	8	0.50	7.5	0.02	7.5	0.39	8	0.02	4.0	0.70	1	0.00	....	0.55	3	0.37	1.0	2.12	1	0.00	....	3.48	1	0.19	2.5	1.29	4
RG‡.....	0.17	4.5	1.45	5	0.00	....	0.97	5	0.36	2	1.67	2.0	0.39	1.0	1.10	4	0.00	7.0	0.28	7	0.00	....	0.53	4	0.21	4.0	1.35	6	0.00	....	2.31	6	0.18	4.0	1.19	5
PB.....	0.35	2.5	2.10	2	0.00	....	1.57	2	0.17	4	1.66	3.5	0.15	2.5	1.27	2	0.04	1.5	0.55	3	0.00	....	0.57	2	0.22	3.0	1.44	4	0.02	....	2.91	3	0.19	2.5	1.44	2
YB.....	0.00	7.5	0.63	7	0.00	....	0.57	7	0.15	5	1.03	6.0	0.11	4.0	0.63	6	0.02	4.0	0.57	2	0.02	....	0.61	1	0.00	7.5	0.35	8	0.00	....	1.20	8	0.04	7.0	0.64	7
RB.....	0.00	7.5	0.31	8	0.00	....	0.28	8	0.06	7	0.50	7.5	0.02	7.5	0.48	7	0.00	7.0	0.11	8	0.00	....	0.24	8	0.00	7.5	0.57	7	0.00	....	1.31	7	0.01	8.0	0.37	8
YG§.....	0.17	4.5	1.71	4	0.04	....	1.18	4	0.13	6	1.12	5.0	0.06	5.5	0.83	5	0.04	1.5	0.33	5	0.02	....	0.37	6	0.13	5.0	1.38	5	0.00	....	3.04	2	0.12	6.0	1.13	6
YR.....	0.46	1.0	2.43	1	0.02	....	1.73	1	0.22	3	1.66	3.5	0.06	5.5	1.22	3	0.00	7.0	0.31	6	0.02	....	0.31	7	0.24	2.0	1.73	2	0.02	....	2.62	4	0.23	1.0	1.53	1
WR.....	0.15	6.0	1.33	6	0.00	....	0.83	6	0.43	1	2.06	1.0	0.15	2.5	1.51	1	0.02	4.0	0.41	4	0.02	....	0.39	5	0.04	6.0	1.55	3	0.00	....	2.34	5	0.16	5.0	1.34	3
Mean rates..	0.21	1.0	1.48	1	0.02	2	1.06	2	0.19	2	1.25	3.0	0.11	1.0	0.92	3	0.02	4.0	0.41	4	0.01	3	0.44	4	0.15	3.0	1.31	2	0.005	4	2.40	1	0.14	.....	1.11	....
Rates of ♂♂..	1.60	1.0	11.27	1	0.15	2	8.07	2	1.44	2	9.58	3.0	0.83	1.0	7.03	3	0.15	4.0	3.15	4	0.08	3	3.37	4	1.14	3.0	10.00	2	0.04	4	18.35	1	1.08	.....	8.50	....

* Fr. = frequency; Rk. = rank. Italic figures indicate rank.

† Cock *I* with flock G.

‡ Hen RG died August 2.

§ Hen YG pecked PB.

TABLE 4

S WHEN ALL 4 COCKS WERE CONTINUOUSLY IN THE PEN OF THE HENS*

(See Table 2 and p. 370)

Treadings		Courtings		"Sex Invitations"		Avoidances		Treadings		Courtings		"Sex Invitations"		Avoidances		Treadings		Courtings		"Sex Invitations"		Avoidances	
Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.	Fr.	Rk.
eriod A (29 Observations between July 4 and August 4, 1940)																							
								♂ IV								♂ III							
																Mean Rates							
.00	7	0.00	7	0.00	....	0.00	7	0.00	.....	0.00	....	0.00	....	33.33	3	0.28	6	1.08	7	0.06	3	1.69	7
.10	6	0.82	6	0.00	....	0.72	6	0.00	.....	6.25	....	0.00	....	50.00	1	0.21	7	1.84	3	0.00	6	2.77	2
.46	3	1.60	2	0.00	....	1.26	4	0.00	.....	9.09	....	0.00	....	9.09	6	0.41	4	1.67	4	0.00	6	1.99	5
.14	2	3.54	1	0.00	....	2.70	2	0.00	.....	0.00	....	0.00	....	37.50	2	0.93	2	2.97	1	0.05	4	2.76	3
.45	4	1.25	4	0.00	....	3.18	1	0.00	.....	0.00	....	0.00	....	31.25	4	0.84	3	1.31	5	0.29	1	2.78	1
.31	1	1.31	3	0.00	....	1.09	5	0.00	.....	6.25	....	0.00	....	12.50	5	1.15	1	2.40	2	0.20	2	2.07	4
.30	5	1.21	5	0.00	....	2.12	3	0.00	.....	0.00	....	0.00	....	0.00	7	0.31	5	1.16	6	0.00	6	1.77	6
.58	2	1.46	4	0.00	....	1.56	3	0.00	4	3.00	1	0.00	....	28.00	1	0.62	.....	1.85	....	0.09	.....	2.33	....
.53	2	6.38	3	0.00	....	6.84	4	0.00	4	2.50	4	0.00	....	23.33	1	2.54	.....	7.54	....	0.40	.....	9.50	....
iod B (34 Observations between August 5 and September 8, 1940)																							
Died								0.00	3	0.00	3	0.00	3	3.31	2	0.56	.....	1.96	....	0.22	.....	3.86	....
								0.00	3	0.00	3	0.00	3	8.57	3	1.95	.....	6.76	....	0.78	.....	13.37	....
od C (31 Observations between September 9 and October 11, 1940)																							
Died								0.08	1	0.10	2	0.04	1	2.44	1	0.05	.....	0.23	....	0.02	.....	2.11	....
								0.48	1	0.60	2	0.24	1	13.97	1	0.30	.....	1.35	....	0.12	.....	12.57	....
k F (48 Observations between July 3 and August 20, 1941)																							
								♂ VII								♂ VIII							
																Mean Rates							
.02	4.0	0.70	1	0.00	....	0.55	3	0.37	1.0	2.12	1	0.00	....	3.48	1	0.19	2.5	1.29	4	0.02	7.0	1.42	3
.00	7.0	0.28	7	0.00	....	0.53	4	0.21	4.0	1.35	6	0.00	....	2.31	6	0.18	4.0	1.19	5	0.09	1.0	1.22	6
.04	1.5	0.55	3	0.00	....	0.57	2	0.22	3.0	1.44	4	0.02	....	2.91	3	0.19	2.5	1.44	2	0.04	2.5	1.58	1
.02	4.0	0.57	2	0.02	....	0.61	1	0.00	7.5	0.35	8	0.00	....	1.20	8	0.04	7.0	0.64	7	0.03	5.0	0.75	7
.00	7.0	0.11	8	0.00	....	0.24	8	0.00	7.5	0.57	7	0.00	....	1.31	7	0.01	8.0	0.37	8	0.005	8.0	0.57	8
.04	1.5	0.33	5	0.02	....	0.37	6	0.13	5.0	1.38	5	0.00	....	3.04	2	0.12	6.0	1.13	6	0.03	5.0	1.35	4
.00	7.0	0.31	6	0.02	....	0.31	7	0.24	2.0	1.73	2	0.02	....	2.62	4	0.23	1.0	1.53	1	0.03	5.0	1.46	2
.02	4.0	0.41	4	0.02	....	0.39	5	0.04	6.0	1.55	3	0.00	....	2.34	5	0.16	5.0	1.34	3	0.04	2.5	1.27	5
.02	4.0	0.41	4	0.01	3	0.44	4	0.15	3.0	1.31	2	0.005	4	2.40	1	0.14	.....	1.11	....	0.03	.....	1.20	....
.15	4.0	3.15	4	0.08	3	3.37	4	1.14	3.0	10.00	2	0.04	4	18.35	1	1.08	.....	8.50	....	0.28	.....	9.20	....

‡ Hen RG died August 2.

§ Hen YG pecked PB.

resulted either from the weight of the cock or coincidentally from a spontaneous reaction by the hen; crouches occurring under these conditions were not considered as "sex invitations." Matings were recorded as incomplete whenever it was obvious that the vents failed to meet.

Although not systematically studied, it was observed in the earlier tests with flock D and other flocks that thirst, food, and roosting drives, especially thirst, seemed readily to take precedence over the sex drive. A cock not watered on a given day was likely to spend much of the observation period drinking, in place of paying attention to the hens. If the observation periods were late in the afternoon, the cock was likely to go to roost with the hens instead of attempting to mate with them. An effort was made to avoid these complicating factors.

COMPARISONS AMONG THE HENS

The analysis in the present section is limited to the relatively simple situation that obtained when the cocks were introduced singly and successively. There was some daily variation in mating frequency by the individual hens. Heuser (1916) made a similar observation. To test for the consistency of matings, the records from April 13 to June 20 of flock D and from April 26 to June 30 of flock F were selected. These data were not complicated by the effect of the presence of more than one cock or by the molting season and were all the more significant, since the time of the year includes the peak of the normal laying cycle. The number of treadings for odd-numbered and even-numbered days were tabulated for each hen, and paired comparisons for all the hens of each flock were then made by the method of "Student" and found to be statistically insignificant (P-values > 0.57),[3] which means that there was no real difference between the number of matings by these hens for the alternate days during the season of the year sampled.

The hens usually paid no attention when one of them was being trod, unless the one being mated cried or struggled vigorously to get free. On rare occasions a hen tried to peck an inferior flockmate while she was being held down by the cock. This was more likely to happen if there was an apparent antipathy between them.

Hen BR of flock D began to molt in July, and her rank in matings dropped from first place (Table 3, mean rate) to second place (Table 4, period A); she continued to mate less frequently as her molt became heavy, and consequently ranked sixth for period B. As a result of BR's decrease in sexual activity and YY's cessation of mating (see below), RY was mated more frequently and rose in mating rank from third place (Table 3) to top rank during period B. About 3 weeks after the close of period C, RY also entered a heavy molt, ceased to mate, and avoided all the males. The molt of the remaining D-hens was light and extended over a longer period of time. The observations of flock F did not extend through the molting season and offered no evidence of its effect on their sexual activity.

The periodicities of the molt and of reproduction appear to be independent (van der Meulen, 1939) but may affect each other secondarily, and their respective cycles may show more or less overlapping. The hens of the flocks that molted heavily were very sensitive to handling, and we received the impression

[3] P-values of 0.05 are taken as the upper limit of statistical significance, the smaller the fraction the higher the statistical probability.

that this hypersensitive condition of the skin contributed much to the tendency of molting individuals to avoid close physical contact with the males.

Hen YY of flock D was originally second in mating rank (Table 3, mean rates) and was first during period A (Table 4), when BR molted. On the first day of period B, YY received a pellet of testosterone propionate, implanted subdermally. Within 3 days she ceased to mate; and after the three treadings that occurred during this transitional period she became lowest in mating rank, remaining so for the remainder of period B.

Inspection of Tables 3 and 4 shows a suggestion of a negative correlation between social ranks and ranking based on the frequency at which the hens were trod and at which they gave the so-called "mating invitation" to the cocks. These and other possible correlations will be considered further on page 380, after other pertinent observations have been presented.

COMPARISONS AMONG THE COCKS

Cocks introduced singly and successively.—As with the hens, the cocks of both flocks showed a day-by-day consistency in their treading frequencies for the period during which this matter was tested with the hens. The *P*-values for the odd-numbered versus the even-numbered days of both groups were greater than 0.58. There was no evidence that molting, which was light in all of the D-cocks, influenced their observed behavior.

A comparison of the cocks is more complicated than that of the hens, as the positive and negative reactions of the hens toward the cocks as individuals may influence, to a greater or less degree, the frequency at which a rooster may have the opportunity to mate, court, receive "sex invitations," or be avoided. The differential behavior of hens toward different cocks was more marked than was the comparable reaction of the cocks to the hens as individuals. Differences between the cocks may be considered in several ways: (*a*) by comparing the total number of matings and other behavior patterns for each cock under like conditions and during equal periods of observations; (*b*) by the examination of the mean frequencies of the matings and courtings of each cock; (*c*) by studying the actions and reactions directed toward each cock by other cocks; (*d*) by a statistical comparison of the frequencies of the female reactions toward each of the cocks; and (*e*) by descriptive remarks on the observed behavior of the cocks.

The recorded treadings by the D-cocks when introduced singly and successively (Table 1), with the possible exception of cock II, showed no very marked differences between them in their success at mating with the hens as a group. Cock II courted the least number of times and was never "invited" to tread. Since some of the D-hens spent much time on the roost, out of contact with certain males, the individuality of the cocks becomes clearer when the rates of male activities are considered as calculated from the time the given cock was closely associated with hens on the floor of the pen. The frequency rates of the males and the mean rates of the females as a group, given in Table 3, suggest greater differences between these cocks than those shown by the raw data.

To test for the statistical significance of the differences between the cocks in the rates presented in Table 3, paired comparisons were made of the frequencies of the behavior patterns of the individual hens when in association with each cock. The mean mating frequency of all the hens when in association with

cock II, of 1.21 matings per hour, is significantly greater than the mean rates of the other 3 cocks (*P*-values: 0.03–0.02). The mean rate (2.17) at which the hens were courted by II was decidedly greater than either of these frequencies with *I* or IV (*P*-values: 0.04 and 0.01). When considered in the different possible pair-combinations, there is no statistical difference between the rates for these types of behavior among the other cocks; differences in the rates at which each of the 4 cocks received "sex invitations" failed to show any statistical significance, in part because the 3 top-ranking hens gave no "sex invitations" during this period. The analysis points to cock II as the most active sexually. His comparative libido contrasts with the fact that he was the only male that all the hens failed to "invite" by crouching.

Statistical tabulations often do not give a complete picture of behavior. This is particularly true if intensities of reactions are considered; hence descriptive remarks have value in the interpretation of the results. Cock II approached the hens quickly or chased them from one end of the pen to the other; this caused them to fly to the roost, where they tended to remain while he was in the pen. With the same total elapsed observation time the females spent a total of 2,322 minutes on the floor with cock II, as compared with 4,926 minutes with cock *I*. Of II's matings, 76 per cent were forced, and none of the hens "invited" him by squatting. Cock *I* approached the hens slowly from the rear and held his head over a female as though ready to grasp her comb, or he raised his hackle; if the hen moved away, he diverted his attention to another; if a hen squatted, he trod. Only 15 per cent of *I*'s matings appeared to be forced; 29 per cent were "invited"; and in the remaining 55 per cent courting and squatting appeared to be simultaneous.

Cocks III and IV appeared to grade between *I* and II as to sex drive but closer to *I* than to II, with III probably more sexually active than IV; both forced 21 per cent of their copulations. In general, this comparison agrees with the preceding analysis.

The negative reactions of the hens toward each of the cocks were not tabulated for flock D. But this factor was considered by an analysis of sustained avoidance made by paired comparisons of the total time these hens spent on the floor with each cock, the *P*-values of which are given in Table 5.

These statistics support direct observations and show that the hens gave significantly more sustained avoidance toward II than toward any other rooster, and the least toward cock *I*. These individual differences also became significant when these same birds were tested in a discrimination cage (Guhl, 1942), where it was found that discriminations were notably influenced by individuality and that the birds acted and reacted according to former pair-contact experience. These observations are in agreement with a conclusion drawn by Tinbergen (1931) that "the members of a flock in which a peck-order occurs know each other individually revealing not only an amazing power of discrimination, but at the same time the working of rather intricate conditioning processes by which an animal forms separately special habits in relation to every individual."

The raw data concerning the mating of the F-males (V, VI, VII, and VIII) given in Table 1 show no marked differences between individuals in total numbers of matings or "sex invitations" received. Cocks VI and VIII courted more and were avoided more than were V and

VII. Some of these differences become more evident when activity rates are compared in a hen-by-hen analysis of the frequency of the female reactions (Table 3) toward each of the cocks.

The following differences have statistical probabilities (*P*-values) that lie between 0.05 and 0.0004. Other possible comparisons are less valid statistically.

TABLE 5

A COMPARISON OF THE TIME WHICH THE D-HENS, AS A GROUP, SPENT ON THE FLOOR IN THE PRESENCE OF EACH OF THE D-COCKS WHEN THE LATTER WERE INTRODUCED SINGLY AND SUCCESSIVELY

Comparison of Cocks	Time (Minutes)*		*P*-Value
	Total	Mean Difference	
I..... 4,926 II..... 2,322		372	0.006
I..... 4,926 III..... 4,325		86	0.003
I..... 4,926 IV..... 4,781		20	†
II..... 2,322 III..... 4,325		286	0.001
II..... 2,322 IV..... 4,781		351	0.004
III..... 4,325 IV..... 4,781		65	†

* The data in the column "Total Time" give the sum of the number of minutes each hen was on the floor with each cock during observation periods. The difference, in minutes, between the cocks is given as the mean difference.

† Denotes a *P*-value of 0.10 or greater.

(It is well to remember that the peck-order of the F-cocks is indicated by the ordinal rank of the numerals and that cock *I* did not meet the F-cocks, and hence his relative social status is not known.) Cocks *I*, VI, and VIII courted more than did V or VII. The hens squatted less frequently for cock *I* than for V, VI, or VIII. They avoided *I* and VI more than VII, and they avoided VIII more than any of the other four males.

Cock VIII had slightly more forced treadings and was less skilful than the others. Cock VII would stand idly, as though oblivious of his female penmates; and VI courted very mildly when a hen came near him. None of these cocks displayed much intensity in sex behavior, and they seemed to be somewhat abnormal, with VIII more nearly approaching the normal pattern in sex behavior. A possible explanation of their apparent abnormality will be suggested later.

As with cock II and the hens of flock D, the cocks that the F-hens avoided at the higher rates (*I*, VI, and VIII) were those that courted with a significantly higher frequency.

Although the F-cocks were relatively weak in sexual initiative, the rates of courting and mating were higher than those found in flock D (Table 3). Each of the following modified factors probably helped produce this result: (*a*) For most of the day preceding his period of free association with the hens each cock was placed in a wire cage in the pen near the hens, to augment sexual readiness. (*b*) The roosts and other possible retreats were blocked off, and the available floor space was approximately halved. (*c*) There was a ninefold increase in the rate of "sex invitations" by the F-hens as compared with those of the D-flock. Confronted by sexually unaggressive males, such reactions by the hens may be classed as compensatory behavior.

Cock *I* of the D-flock was included in tests with F-hens in an attempt to secure a better basis for cross-comparisons of the mating behavior of the two sets of hens. The utility of cock *I* for comparative purposes is marred by complications. In addition to the differences just outlined, this cock was now a year older; he was not penned with the F-cocks but had a pen to himself; he was now placed

with young, rather than aged, hens; and these younger birds, between days with cock I, associated with the relatively unaggressive F-cocks. Contrariwise, between periods of association with cock I, the D-hens had experience with the notably aggressive cock II. Thus, several elements of both the physical and the social environment differed in the two sets of tests.

We received the impression that cock *I* was relatively less acceptable to the F-hens than was to be expected from his comparative showing with the D-cocks.

There was no discernible modification of the earlier sex technique shown by *I* in the later situation. He courted and trod the F-hens at a heightened rate, indicating that the generally increased frequencies of these behavior patterns among the F-hens resulted from altered conditions or modified traits of the hens.

With both flocks D and F the observations made when cocks are introduced singly and successively into the pens with hens indicate that the social position and the sexual drive of the cocks were not necessarily related and that the reproductive efficiency in the flock was only partially determined by the relative libido of the cocks. However, when 4 cocks were placed with a small group of hens, the interrelationship of their relative social aggressiveness and their sexual drive became apparent.

Four males in the pen of the hens.—When all 4 D-cocks were placed in the pen of the D-hens, the male interaction became intense, and for 11 days the hens spent most of their time on the roost. The observations were divided into three periods (Tables 2 and 4). Period A, of 29 observations, may be considered as transitional; period B, of 34 observations, as a climax; and period C, of 31 observations, as a test. Cock IV died accidentally on July 24, shortly before the end of period A.

The previous male peck-order, with the cocks arranged in the straight line of *I*, II, III, and IV, changed on June 28, when IV defeated III. Cock II pecked III in the eye; and as a result of this temporary visual handicap, cock III lost a contest with IV and consequently his social position. He remained on the roost and lost 13 ounces in weight; by the very end of period A he ventured to the floor to feed, but then only when *I* and IV went to the roost. His hunger dominated his sex drive almost to the exclusion of the latter. Cocks II and III developed a toleration for each other on the roost, where they spent much time together. The sexually active II became conditioned to ignore the hens by a slower process. The alpha cock, *I*, charged at him when he approached a hen and drove him to the roost. This caused all the hens to scatter hurriedly and fly to any perch. Cock II soon learned to pass more time on the roost, and the hens learned to run whenever he came to the floor to feed. He lost 6 ounces in weight, and his hunger drive approximated that of III. Of II's 13 matings, 12 were incomplete (the vents did not meet), as a result of the resistance of the hens or the interference of *I* and/or IV.

The intense antagonism of *I* toward II contrasted with *I*'s curious and extreme toleration of IV; the latter would interfere even when *I* was in the act of treading a hen; IV did so 30 times, while *I* interfered with 20 of IV's matings. The hens learned to avoid IV, and it appeared as though they might nullify his sexual activity by means of their negative discriminations; he succeeded in obtaining 15 of his 33 copulations by pushing *I* from treading position and mounting immediately. At first *I* would peck

his usurper, but later he showed no agitation. This unusual toleration probably facilitated IV's successful revolt over II, which occurred the day before IV died.

During period B both II and III spent more time on the floor, but they were conditioned to remain at a distance from the alpha cock and the hens. Cock II still courted at intervals, and his single copulation occurred early in this period. Cock III was psychologically castrated, i.e., completely suppressed sexually; and II nearly so. Cock II crowed at times, but III was not heard to crow after he lost his social position. The size of comb did not fluctuate to any marked degree in any of the males during the time when some were sexually suppressed. A simple test was made to determine whether the frustrated cocks II and III were capable of sexual behavior. Cocks *I*, II, and III were each placed singly on two different days with a strange flock for 30-minute periods. Cock II trod 7 times and courted 27 times; III trod 5 times and courted 40 times, which compared favorably with *I*'s 6 matings and 41 courtings under the same conditions. However, when II and III were then exposed singly for 30 minutes with the D-hens, neither of them trod, and courtings were limited to 2 wing-flutters by III. This indicated that their sexual inhibition depended on their associates and was definitely related to the hens of the home pen.

The dominant cock, *I*, was removed during period C to note whether the sexual activity of the inferiors would become re-established. Cock *I* was placed with flock G. Over an interval of 33 days cock II was seen to tread 3 times (2 were out of the formal observation time), and III mated 11 times (7 out of the formal observation period). All of II's copulations were forced, but III trod only on invitation. Cock II pecked III when the latter trod, and there was some indication that II might in time suppress III completely. Both cocks would at times give the food-call; but when a hen responded, they would peck her. When these results are considered with those of the partial control situation summarized in Table 1, the slight difference (in period C over B) in sexual behavior appeared to be negligible and suggests either that the conditioning effects associated with psychological castration tend to persist or that the continuing conditioned avoidance displayed by the hens toward these roosters may have been a factor in the failure of cocks II and III to copulate at more nearly a normal rate.

Another test was made to determine the nature, if any, of heterosexual conditioning involving the frustrated males. The alpha cock, *I*, was used as a control to evaluate, in a general way, the relative sexual behavior of the hens. The cocks were exposed to flocks D and G singly on alternate days; each day one cock was placed with the D-hens and another with the G-hens for 20 minutes. This rotation continued until each rooster had 8 exposures to each group of hens; between tests these males were housed together in a separate pen. The results are given in Table 6, from which it is evident that the inhibited sexual activity of cocks II and III when with the D-females affected their behavior when with the hens of flock G. Their courting increased notably, although they were not very successful in mating. The evidence supports the previous test, which showed that these two roosters were conditioned not to approach the D-hens. An analysis of sustained avoidance indicates that the D-hens were also conditioned to avoid these particular cocks.

The observer gained the impression

that the G-hens also learned to spend more time on the roost when cocks II and III were introduced—an impression that was confirmed in part by statistical analysis, since hens of flock G spent 37 per cent less time on the floor with II than with *I* (*P*-value, 0.03) and 25 per cent less time with III than with *I* (*P*-value, 0.07). The D-hens spent 34 per cent less time with II than with the alpha cock (*P*-value, 0.005) and 40 per cent less with III than with *I* (*P*-value, 0.006). Here, as in the discrimination tests (Guhl, 1942), the hens as a statistical group showed differential behavior toward the cocks as individuals.

We turn now to consider the F-males, which, as has been previously stated, were rather weak, so far as intensities of reactions were concerned, in both their social and sexual behavior. When all 4 were placed in the pen of the F-hens, there was an increase in pecking frequency among these cocks; but the suppressive influence of low status in their peck-order was less pronounced than

TABLE 6

SUMMARY OF THE SEXUAL BEHAVIOR OF THE D-COCKS WHEN TESTED SINGLY AND ALTERNATELY WITH THE HENS OF FLOCKS D AND G

Peck-Order	With Flock D		With Flock G	
	Treadings	Courtings	Treadings	Courtings
I	9	54	21	93
II	0	19	3	126
III	0	2	3	95
Total	9	75	27	314

with cocks II and III in flock D. Cock VII approached the psychological castration stage, as he trod only 7 times (Table 2), 4 of these times resulting in incomplete copulations. The differences between the frequencies of VII's matings and courtings and those of the other three F-cocks were statistically significant (*P*-values ranged from 0.03 to 0.01). There were no significant differences between these records of the other three males.

TABLE 7

SUMMARY OF THE INTERFERENCES WITH MATING OR ATTEMPTS TO MATE AMONG THE F-COCKS

The figures present the number of interferences by each of the cocks listed on the left with attempts to copulate by each of the other cocks.

Peck-Order	V	VI	VII	VIII	Total
V	.....	55	7	57	119
VI	17	.....	2	9	28
VII	0	0	.....	0	0
VIII	13	1	0	.....	14
Total	30	56	9	66	161

Interferences with mating or attempts to mate are summarized in Table 7. Only the frustrated cock VII was completely inhibited from taking an aggressive role in competition for the possession of a receptive hen. These attacks were not limited to cocks of a particular status, although the alpha cock interfered in about 74 per cent of the observed instances. During the early part of this 48-day period these obstructions to copulation were caused by pecks delivered on the treading male; by the fourth week the pecks became very light, or the interfering male would merely approach the mating pair; and by the fortieth day there was a tendency for the interloper to ignore the copulating male and to grasp the comb of the hen being trod.

When an inferior rooster crowed or gave the food-call, he was usually challenged by the alpha V, with the result that such vocalizations disappeared

during most of these weeks. The male interaction lessened in intensity after the twenty-fifth day, and both these calls then reappeared to some extent. The hens showed a tendency toward sustained avoidance of the partially frustrated males by moving to neutral corners. This female reaction could not be qualified to the same extent as with the D-hens, since the roost was screened off and the floor space restricted during observations to one-half that of the normal pen. With such closer contact one might have anticipated greater interaction between the cocks and more evidence of psychological castration among normal young cocks.

At the end of the observations correspondence with the breeder who furnished the birds revealed some interesting information on the early history of these cocks. Cocks V, VI, and VII were part of a group of 24 that had been penned with about 200 hens and were shipped to us as culls, which suggests that they were probably inferiors in this group of cocks. The indications are that they were conditioned when received, as 4 of the original 24 cocks had been killed by their superiors. Little was known about cock VIII; he came from a different source. If this assumption is correct, one might conclude, as with the D-cocks, that conditioning of mating behavior patterns in roosters, once formed, tends to persist.

The data for the cocks do not show any strong correlations between the social position per se of the individual males and the frequency of their mating behavior when introduced singly and successively into a pen of hens ($r = 0.4$; P-value > 0.1). Cocks tended to be consistent in the frequency of matings when not inhibited by the presence of other males. However, as has just been stated, when 4 cocks were placed together in a pen containing a small number of hens, a form of suppression developed among the cocks; and the dominant male almost completely castrated psychologically some of the low-ranking roosters. This shows that, as a result of their greater freedom of the pen, the dominant cocks possessed precedence to mating in addition to precedence to food. There is some evidence that the activities leading to the frustration associated with sexual suppression, or the suppression itself, although it does not incapacitate a cock sexually, conditions the hens to avoid a suppressed cock or modifies his behavior in a manner which makes him less acceptable to the hens.

SEX DIFFERENCES IN BEHAVIOR

The males are more aggressive than the females and tend to avoid other males strongly or to fight back rather than to submit to them, whereas the hens with less aggressiveness than the males submit more readily to cocks and to aggressive hens by lowering the head or by squatting. Like the cocks, they also frequently avoid social contacts. The peck-orders of cocks are somewhat unstable, and those of hens are relatively stable (Sanctuary, 1932; Masure and Allee, 1934). An alpha hen, "antipathies" aside, becomes tolerant of low-ranking individuals. This reduction of social interaction between individuals of widely separated ranks becomes greater between the males and the females, with the consequence that males normally do not peck females but tolerate them; and the females show no marked avoidance of normal males, so that typically two essentially unisexual peck-orders occur in a flock composed of several cocks and several hens. Carpenter (1942) has described a similar situation in his hordes of rhesus

monkeys on Santiago Island off the coast of Puerto Rico. In a flock of intersexual Brown Leghorns, Domm and Davis (1941) found that, in general, the most masculine birds were at the top, and the most feminine were at the bottom, of the ranking among the males. The hens appear to learn readily that cocks will not peck them, although they also learn to avoid a rough and sexually active cock such as II.

INTERSEXUAL BEHAVIOR

Hens secrete androgens, as well as estrogens, as shown by comb regression to the capon level in bilaterally ovariotomized hens (Domm, 1927). Koch (1937) has demonstrated by results obtained by applying the hormone to the surface of the comb that comb size can be used as an indication of the amount of androgen present. A marked correlation exists between aggressiveness and comb size in normal or in testosterone-injected hens (Collias, 1943). We have records of pronounced masculine behavior in normal hens that merit brief attention. In a previous report (Guhl, 1942) mention was made of 3 hens that wing-fluttered to other hens, and this reaction has since been observed under somewhat similar conditions in other flocks. In a more recent experiment the food-call appeared to be rather common among some hens, whether in the pen, out of doors, or in isolation. The crowing of hens is a well-known phenomenon. In addition to GG of flock D, 5 normal hens of a different flock than those mentioned here have been heard to crow; one of these (RG) crowed quite regularly, whether in isolation or in the pen with others, but in the latter case only when she was the alpha bird.

We have a record of 20 unisexual matings in which 3 normal hens (none of which were in the flocks discussed in this paper) took the male role with 6 different hens. None of these had received experimental treatment with hormones. The vents are known to have met in 10 of these cases. Observations were facilitated by the fact that the feathers in the region of the vents were lacking. Treading movements with the feet were noted in 9 cases. In nearly all these instances, the hen that took the normal role of the female squatted submissively before the threatening superior before the latter mounted. When one hen (GB) trod, she everted her cloaca; and on two occasions when the vents failed to meet, she discharged a stream of rather clear fluid. None of the treading hens had been heard to crow. All 3 hens assuming the male position were either laying or laid within 4 days of these pseudo-matings, and all were socially dominant over the hen that was trod. No cocks were kept with these flocks.

Heuser (1916) reported a case of a hen that attempted to mate 35 times, "in as perfect a manner as the best male," with 13 different individuals. This, he stated, occurred at times of egg production and nonproduction, mating and nonmating, and while the males were present or absent. Heuser kept no records of social position; hence we do not know the relative social dominance of his malelike hen. More observations are needed before a complete behavioristic interpretation can be attempted, but these cases do raise the question of how widely the capacity for male copulatory behavior is distributed among otherwise normal hens and also what social and other conditions are required to elicit this response in a female. Whitman (Carr, 1919) noted that the potentialities of male and female sexual behavior patterns in pigeons were almost on a par in the two sexes, and he dis-

cusses some of the conditions under which either males or females may exhibit activities peculiar to the opposite sex.

Androgen-treated hens became highly aggressive, and their female penmates learned to avoid them rather than to submit. Since the submissive squat appears to be essential to copulation, the treated hens in our flocks did not secure the opportunity to display the male copulatory pattern if it was induced. In the cases in which we saw unisexual mating, the treading hen, although the dominant of the pair, did not peck hard or often; instead she frequently elicited submission rather than avoidance. Since individuals develop special habits in relation to every individual (Tinbergen, 1931; Guhl, 1942) and since, as discussed by Allee, Collias, and Lutherman (1939), social inertia may enforce or replace aggressive behavior patterns, it appears logical to assume that habit may be important in the patterns of sex behavior shown by hormonally treated fowl. For example, when YY received a pellet of testosterone propionate, her aggressive behavior was noted in the discrimination pen (Guhl, 1942) long before it became evident in her home pen; and when she was placed in the pen of flock G for a test, she drove all these hens about their pen, but on return to her own flock she assumed pair-behavior patterns in accord with her social status of that day (cf. Douglis, 1944). The same dominance of learned behavior over physiological state was shown by cocks II and III, which, although capable of copulation, did not mate with the D-hens even when the suppressive influence of cock *I* was removed. Allee, Collias, and Lutherman (1939) found that low-ranking hens treated with androgen not only rose in the social order but maintained their new social status long after injections had ceased and the recipient hens had lost their androgen-mediated aggressiveness.

CORRELATIONS BETWEEN OBSERVED RANKINGS AMONG THE HENS

Before summarizing observed correlations, it is necessary to pause briefly to introduce pertinent background material about flocks A6, D_{1939}, and E. The records of these last three flocks were obtained during a total of 32 hours of observations on flock A6, 35 hours on flock D_{39}, and 8.5 hours on flock E, all during the first 8 months of 1939. Observation periods were 10 or 15 minutes in length and usually occurred late in the afternoon. Flocks A6 and E were composed of 6 hens; D_{39}, of 7 hens. The basic relations are summarized in Table 8.

There was a pecking triangle in flock A6 at this time; a linear pecking order existed in flock E and during about half of this period for flock D_{39}. Two reversals of social position occurred in flock D in the second half of the period, but these did not concern the two top birds; the social order indicated in Table 8 represents the earlier situation.

The data from the 1939 observations are somewhat complicated as a result of hormone treatments given in connection with other experiments. The treatments consisted of injections of estradiol benzoate or of thyroxin into birds selected from various social levels and, except for the higher doses of thyroxin, produced little or no effect on the rate of mating. All data are omitted from Table 8 that were recorded at the time when some of the top birds (2 hens in flock D and 1 in flock E) received doses of thyroxin sufficient markedly to depress their frequency of mating.

Mention was made previously of a tendency toward a negative correlation

between the social position of the hens in flocks D_{1940} and F and the rates at which they were trod or gave the sexual squat. In the more careful statistical study of these matters it was decided to concentrate on data secured when each cock was with his respective flock of hens singly and successively. The presence of 3 or 4 cocks in the pen at the same time introduced complications that we have been at some pains to describe. So far as flocks D_{40} and F are concerned, the first correlation studies are based on data given in Table 3; the relations in the other flocks have just been given in Table 8. The correlation coefficient (r) was calculated from the regression formula

$$r_R = \frac{\epsilon v_1 v_2 - \overline{V}\epsilon v_2}{\epsilon v_2^2 - \overline{V}\epsilon v_2}.$$

Further, since social status is given here summarily in terms of rank achieved rather than by listing in detail the pecks delivered and received, it is probably best to examine the correlation of social ranks with rankings based on the rates for other observed activities.

We are interested in the correlations within each flock considered as a unit and within various flock combinations. The combined correlations given in Table 9 are for the 1940 observations on D_{40} and F; on the 1939 observations on A6, E, and D_{39}; and on all the flocks considered together. In this last instance the D-flock for both years was considered as one unit and given the rank-order based on mean rate of performance. The comparison of correlations between flocks that differ in size was accomplished by the simplest-possible treatment. The top-ranking hens were regarded as equivalent; so were those in bottom rank; the intermediate ranks were adjusted by simple "stretching" of the smaller number of ranks to give them their appropriate place in the larger series. The correlations are listed in Table 9, with the statistically significant values shown in italic type.

Negative correlations.—Although not always statistically significant in all flocks, there were negative correlations between ranks based on frequency of mating in relation to position in the

TABLE 8

SOCIAL POSITIONS OF THE HENS AS RELATED TO THEIR FREQUENCY OF MATING AND "SEX INVITATIONS" (SEE P. 370)*

Observations made on 3 flocks during 1939

Social Position	No. of Treadings			No. of "Sex Invitations"		
	A6	D_{1939}	E	A6	D_{1939}	E
1	5	30	0	0	14	0
2	.....	38	0	.....	18	0
3	9, 77, 40†	39	32	0, 27, 6†	10	27
4	.....	46	31	.....	32	10
5	.....	93	13	.....	43	10
5·5	30, 37‡	.....	.....	5, 0‡	.....	.....
6	.....	66	4	.....	43	1
7	.....	82	.....	.....	51	.....

* Blank spaces indicate that there were no hens at these levels in the social order. Half-steps in social position mark deviations from a straight-line peck-order.

† Members of a pecking triangle.

‡ Two hens tied for the omega position.

peck-order: the top-ranking hens were trod less than their subordinates. These results are statistically significant for the three flock combinations and for D_{39}.

The correlations between social rank and the frequency of giving the sexual crouch (so-called "sex invitations") were also steadily negative. They were statistically significant with the D-flocks for both years and when all observations for 1939 (A6, F, D_{39}) are considered together. Although still negative, the value of r for

TABLE 9

CORRELATION BETWEEN RANKINGS AMONG THE HENS

Statistically significant correlations are given in italics. (D_{39} and D_{40} signify the D-flock for 1939 and 1940, respectively.)

Flock	No. of Pairs	Correlation	*P*-Value*
	Social Rank : Mating Rank		
A6	6	−0.2857	>0.1
E	6	−0.3642	>0.1
D_{39}	7	*−0.8929*	*<0.01*
D_{40}	7	−0.6785	<0.1
F	9	−0.4000	>0.1
D_{40}, F	16	*−0.5264*	*<0.05*
A6, E, D_{39}	19	*−0.5427*	*<0.02*
All	28	*−0.6407*	*<0.01*
	Social Rank : Rank in "Sex Invitations"		
A6	6	−0.1613	>0.1
E	6	−0.5757	>0.1
D_{39}	7	*−0.8909*	*<0.01*
D_{40}	7	*−0.7600*	*<0.05*
F	9	−0.0833	>0.1
D_{40}, F	16	−0.2677	>0.1
A6, E, D_{39}	19	*−0.6033*	*<0.01*
All	28	−0.3210	<0.1
	Rank in "Sex Invitations" : Mating Rank		
A6	6	+0.7714	>0.1
E	6	*+0.9740*	*<0.01*
D_{39}	7	*+0.8214*	*>0.02*
D_{40}	7	*+0.8909*	*<0.01*
F	9	*+0.8666*	*<0.01*
D_{40}, F	16	*+0.8213*	*<0.01*
A6, E, D_{39}	19	*+0.5185*	*>0.02*
All	28	*+0.5640*	*<0.01*
	Social Rank : Courting Rank		
D_{40}	7	*−0.9286*	*<0.01*
F	9	−0.4622	>0.1
D_{40}, F	16	*−0.6794*	*<0.01*

* The *P*-values are based on Fisher (1936, p. 202).

TABLE 9—*Continued*

Flock	No. of Pairs	Correlation	*P*-Value*
	Courting Rank : Mating Rank		
D_{40}	7	+0.6071	>0.1
F	9	*+0.7500*	*0.02*
D_{40}, F	16	*+0.5465*	*>0.02*
	Rank in "Sex Invitations" : Courting Rank		
D_{40}	7	+0.6429	>0.1
F	9	+0.3109	>0.1
D_{40}, F	16	+0.4629	>0.05
	Courting Rank : Avoidance Rank		
F	9	+0.1167	>0.1
	Mating Rank : Avoidance Rank		
F	9	−0.3000	>0.1
	Rank in "Sex Invitations" : Avoidance Rank		
F	9	−0.5667	>0.1
	Social Rank : Avoidance Rank		
F	9	−0.0333	>0.1

flock F is very low. The D_{40} hens gave no "sex invitations" to cock II and very few to cock III.

The correlations between social rank and rank based on the frequency at which the hens were courted by the cocks are also steadily negative, significantly so for D_{40} and for D_{40} plus F. While statistically not significant, the negative correlations between mating and avoidance ranks and between those of "sex invitations" and avoidance are in accordance with expectation. The insignificant correlation between social and avoidance ranks shows that in flock F, the only one for which data are available, the social independence of the top-ranking hens included a strong tendency not to avoid the cocks. These observations do not give a basis for predicting what the behavior of the F-hens might have been in the presence of cocks with more highly developed sexual initiative.

Positive correlations.—The correlations between "sex invitations" and matings were uniformly high and were statistically significant except for flock A6. The correlations between courting and mating ranks were also all positive and were statistically significant for the F-hens and for F and D_{40} flocks considered together. The similarly positive correlations between "sex invitations" and courting lack statistical significance, as does also the low correlation between courting and avoidance.

Probably the most interesting of these correlations is the inverse relationship between high social rank and frequency of being trod by the cock. Our data on this point were obtained independently by two observers and are supported by certain observations in the mating pens. As Tables 3 and 8 show, in flocks A6, D (1939 and 1940), and E the alpha hens were trod at the lowest rate. The bird in flock A6 for which only 9 treadings were observed was formerly the top hen in this flock, although at the time of this experiment she was tied for third place with two other hens.

The behavior of GG, in 1939 the alpha hen of flock D, is also illuminating. This hen was at that time the least trod of her flock, "invited" the cock to tread the least, laid well in comparison with the other hens, was a good fighter in staged encounters with strange hens, and the cock frequently courted and waltzed around her. Hen GG also displayed some cocklike behavior: when the cock wing-fluttered to her, she would reciprocate by waltzing around him, giving a rudimentary male-behavior pattern; at times she pecked at him. Only GG among the normal hens of flock D was heard to crow or seen to wing-flutter in the pen. When GY and BB were transferred to flock D in 1940 from flock A6, where they were at the bottom of the peck-order, these hens moved to the top of the new hierarchy and replaced GG at the bottom of the treading order (Table 3).

Although we have not attempted to make a similarly elaborate statistical analysis of the observed correlations when all cocks were placed together with their respective flocks of hens, we have compared the ranks based on the behavior when each cock was introduced singly (Table 3) with that shown when all were present together with the hens (Table 4). The social ranks remained constant in both flocks D_{40} and F—the two for which data are available—during the entire period under discussion. Hen GB, of flock F, died before the second set of observations (those recorded in Table 4) were begun; but her death merely caused YR and WR to become Nos. 7 and 8, respectively, in the peck-order, rather than Nos. 8 and 9.

All the calculated correlations between ranks based on mating, courting, so-called "sex invitations," and avoidances, with 1 cock present, as compared with the same reactions with 3 or 4 present, are positive. The only correlation that is statistically significant is that regarding treading ranks under the two sets of conditions. Here $r = +0.8055$ with a P-value of <0.01. The ranks based on courting show that $r = +0.3711$ ($P > 0.1$), and for "sex invitations" $r = +0.4343$ ($P > 0.1$). Data on avoidances are available for flock F only, and there $r = +0.3095$ ($P > 0.1$).

It is possible to subject the available data to various other statistical analyses. These are all that have seemed profitable for us to discuss. We trust that the data are given in sufficient detail so that anyone interested may make such statistical and other tests as meet his needs.

DISCUSSION

In order to discuss the detailed observations more intelligently, it may be well to pass in review, in summarized form, the correlations that we consider to be fairly valid, or at least suggestive, when all our data and experience are considered. For the hens these are presented in the following informal tabulation.

I. NEGATIVE CORRELATIONS

1. Social position : frequency of mating
2. Social position : frequency of being courted
3. Social position : frequency of "inviting" the cock to mate

II. POSITIVE CORRELATIONS

1. Frequency of giving the sexual crouch : frequency of mating
2. Frequency of being courted : frequency of mating

In addition, there were suggestions of a positive correlation in each test made between frequency of being courted and the frequency of giving "sex invitations." All these correlations are consistent wherever cross-checking is possible.

The interesting negative correlation between ranks based on frequency of mating and social rank requires further discussion. Our results at this point are to be contrasted with those of Skard (1937), who obtained a positive correlation of 0.53–0.55 (P-value about 0.05) between mating "preference" and the peck-order with one cock and a statistically insignificant positive value of only 0.24 with the same cock in another flock of hens. The performance of a second cock, Skard says, "does not show any conformity with the first cock's preferences."

Skard obtained her "mating-preference" order (*a*) by summing all the approaches of her cocks to the different hens or (*b*) by considering weighted values for different recognizable aspects of male-initiated sex behavior, with a value of 1 for each "weak impulse" and of 6 for each copulation. Skard's recorded data do not indicate either the sign or the degree of correlation that she found for either sex between rank in the peck-order and the number of actual copulations; hence they are not directly comparable with our results.

Murchison (1935*b*) reports a positive correlation between the number of times a hen was trod and the distance she traveled toward another hen in his "social reflex runway." The hen that traveled farthest had the highest frequency in mating. He found also (1935*a*) that the hen traveling the greater distance in his special runway dominated the other of the contact-pair in physical combats. Hence, in our terminology,

Murchison found a positive correlation between rank based on the number of times a hen is mated and her rank in the peck-order of the female flock. He observed 292 copulations between 5 pullets and 3 cocks and gives no statistical analysis. An examination of his published records, using the statistical methods we have applied to our own data (in which we based mating rank on the total observed treadings for each hen) indicates a positive coefficient of correlation (r) of 1.0 when 0.9587 would have a statistical probability (P-value) of 0.01. Pertinent differences of Murchison's (1935*b*) methods of observation from ours are as follows: (*a*) Observations on mating were made when his birds were 16–48 weeks old. (Sixteen weeks is an unusually early age at which to expect sexual maturity.) (*b*) The dominance relationships among the pullets were determined in the "social-reflex runway" when they were about 32 weeks old, or 16 weeks after observations on matings began. (*c*) The mating data obtained when cockerels were used singly and successively were pooled, without distinction, with those recorded when all the males were penned with the pullets. (*d*) Murchison does not describe the cage in which the pullets were housed with the cockerels. We have, therefore, no way of knowing whether the matings Murchison observed are comparable with ours, made under other conditions and on a different breed.

In our flocks the over-all correlation between social rank and ranked frequency in mating is regularly negative rather than positive—and significantly so, according to accepted statistical standards, when larger numbers of comparisons are made. It is interesting to note that Schjelderup-Ebbe (1935), whose observations we have often verified, also reported that his top-ranking hens mated less frequently than those with low social status. Schjelderup-Ebbe presented naturalistic observations only without supporting statistical analysis.

We can make some tentative suggestions concerning the important discrepancy between our observations, together with those of Schjelderup-Ebbe, and the findings of Murchison and probably of Skard. We know that differences in ancestry, in previous history, in housing, and in treatment often produce important differences in behavior. Hens high in the social order have greater freedom of movement. Such freedom can be important in allowing more frequent meeting of dominant hens with somewhat sluggish cocks; and Murchison's cocks may all have been sluggish, since 3 of his 6 cocks did not mate. Contrariwise, a negative correlation between mating and social rank may well be the normal relationship when cocks show the usual amount of sexual initiative. Under such conditions the greater tendency of hens low in the social order to give the submissive sexual crouch did much to determine mating frequency. Skard's experience (1937), like ours, shows that there are differences between flocks even under similar experimental conditions; and doubtless there are still greater differences under the divergent conditions used by different investigators. Perhaps there are differences between breeds.

The situation is not simple and becomes less so when other social relations are considered. Despite the advantage of a high social level of aggressiveness in competitions for food and space, beyond some undefined point, female aggressiveness appears to interfere with the mating process. Also, beyond a considerably higher level, aggressiveness of a cock toward associated hens—cock II, for example—lessens his chances for mating.

When different cocks were introduced singly and successively into the pen of the hens, individual differences were revealed in courting and mating habits and in the relative frequency of these activities. The individual differences, though sometimes statistically valid, were not startling until after the cocks had been placed together simultaneously with the hens. Then followed, especially with flock D, a severe conditioning of certain subordinate cocks. This was continued until the individuals were sexually suppressed so far as these hens were concerned, even in the absence of the alpha cock. The suppressed males were less effective even when they were introduced singly into a flock of strange hens (Table 6).

Heuser (1916), who did not describe peck-orders among his birds, found evidence of sexual suppression among males that he related to constitutional vigor. He stated: "It is generally believed that males of high constitutional vigor will overcome the low vigor birds and prevent their mating. However this is not always true, as shown by the behavior of 'black,' one of the low vigor birds, that had third highest number of matings although chased and bullied by all the other males in the pen." This suggests a peck-order relation to what he calls "constitutional vigor." If one could assume that rank in vigor is correlated with social position, one could find parallels between his results and those presented in this paper, such as: (*a*) "high-vigor" males mated more frequently than medium or "low-vigor" males; (*b*) "low-vigor" females mated, on the average, more frequently than hens with high "constitutional vigor"; and (*c*) the correlation between "constitutional vigor" of the females and mating power is weaker than with the males; it is also negative with hens and positive with cocks.

Murchison (1935*b*) found that 3 of his 6 cocks did not mate with the hens with which all of them were associated. This may have been an unrecognized instance of sexual suppression similar to that shown diagrammatically in our 1940 D-flock. In his flock, the alpha, beta, and omega cocks did all the treading. In our flock D, males with intermediate ranks also were suppressed. We found evidence of so-called "favoritism" between cocks *I* and IV. There is no recorded reason why Murchison's omega cock trod hens when those in third, fourth, and fifth ranks did not.

Our observations on domestic fowl under laboratory conditions may be compared to some others made on another gallinaceous species in nature. Simon (1940) and Scott (1942) studied the mating behavior of the sage grouse (*Centrocercus urophasianus*) and found basic behavior phenomena that bear rather close resemblance to those of *Gallus domesticus*.

During the breeding season the cocks of the sage grouse develop a dominance organization composed of a master-cock, a subcock, 3–6 guard cocks, and a variable number of "outside" or isolated cocks. A formalized "wing-beating" constitutes the chief manner of fighting between the males. The hens are more passive but may peck other females. Strutting increases in the presence of females, and mating by inferior males appears to stimulate an attack on the copulating cock. The hens initiate coition by a squatting reaction in front of a cock. Satiation on the part of the master-cock may cause receptive hens to crouch before a subcock or guard cock (Scott, 1944). At daybreak and again during the late afternoon the cocks gather at their strutting grounds and arrange themselves in a number of dominance orders. The hens enter the assembly later and

appear to avoid a poorly integrated group of cocks engaged in much fighting. In these particular observations the master-cocks were involved in 74 per cent of the matings, the subcocks obtained 13 per cent of the copulations, and the guard cocks 3 per cent. The more numerous isolated cocks at the edge of the breeding area secured less than 10 per cent of the total matings observed. It should probably be noted that neither Simon nor Scott stated that the observed cocks were marked and recognized as individuals. These conditions strongly resemble the situation that we have described for flock D_{40} when all cocks were present together.

Skard (1937) was apparently impressed by the so-called mating preferences in her flocks. Warren and Kilpatrick (1929) found evidence of "preferential mating," since three of their hens did not mate with one of the cocks although they did with others introduced alone into the pen. Almost all students of the social behavior of the domestic fowl, from Schjelderup-Ebbe (1922) on, have seen reactions that can most briefly be characterized by the anthropomorphic terms of preference and its opposite, antipathy. We have not yet had better illustrations of such reactions than the antagonistic behavior of cock *I* of flock D_{40} toward cock II or by his weak reaction ("preferential behavior") to the aggression of the otherwise well-subordinated cock IV.

We found little evidence that the males which we observed gave marked positive or negative attention to particular hens. As a rule, the gradation was fairly gradual from the most- to the least-trod hen. Exceptions did occur. For example, when introduced singly, cock II mated with RW at the rate of 2.40 times per hour and with BR 2.20 per hour, but with the hen third in mating frequency, at the rate of only 1.25 per hour. Although cock II courted GG at a fairly high rate, he mated with her infrequently. Cock III copulated much more frequently with BR, cock V (flock F) with YR, and cock VIII with YR, than was to be expected on the basis of their treading of other hens. Cock *I*, when with the F-hens, may have avoided mating with YB and RB. When 4 cocks were together in the flock at one time, *I* and IV mated more with one individual (YY) than with any of the others.

These particular instances of mating "preferences" or "antipathies" may have been the result of the small sample rather than a reality; it would not be surprising if they were real. On the other hand, these differences in the rates at which a given male mates with several hens may be as much a matter of the reactions of the individual hens toward the male as of the behavior of the cock toward the different hens. Some evidence in this regard is fairly apparent in Table 8 and in the avoidance shown by the hens of flock D_{40} to cocks II and III in their later association with those sexually suppressed individuals. However, an adequate discussion of this phase of mating behavior would lead us beyond the scope of the present report.

Finally, what is the evidence that the social position of an individual or its sexual-behavior complex may affect its reproductive success? It is obvious that a socially suppressed cock loses much of his opportunity for mating. It is probable that sexual suppression of a cock need not be carried to the point of psychological castration to produce a reduction in the number of his progeny. Crew (1926) and Warren and Kilpatrick (1929) report that the competitive efficiency of sperm may be of relatively short duration (about 3 days) and that sperm of a new

male may supplant those of a preceding mate. Malbandov and Card (1943) found that eggs fertilized by stale (senescent) sperm show an increase in failure to hatch in proportion to the age of the sperm, although no significant differences in embryo mortality occurred until the sperm were more than 10 days old. They concluded that "it is possible that unpopularity of individual hens with males and insufficient number of males may cause some hens to lay fertile eggs that are incapable of hatching because they were fertilized by stale sperm." Fertilization by artificial insemination depends, among other factors, upon the concentration of sperm (Munro, 1938; Hartman, 1939). Kosin (1944) demonstrated that the numerical relationship between functionally active germ cells and those functionally inactivated by X-rays determine the proportion of eggs fertilized by the specimen of semen treated. Parker and Spadden (1943) found that inanition decreased the semen volume, the number of spermatozoa per collection, and the fertilizing capacity of the males.

Lamoreux (1940) in a study of infertility of fowl lists, among other factors, some causes of infertility that may be influenced by heterosexual behavior, such as "preferential mating," number of matings, and the time elapsed since last mating. All the evidence at hand indicates that these factors may be influenced by the individuality of the cocks and, to a lesser degree, by that of the hens. They are also affected by the learned pair-reaction patterns of the hens and the cocks.

The already complicated problem is rendered still more complex by indications that in the domestic fowl the competition between cocks for mates does not end when a given cock is successful in matings. The sperm from successive matings by two individuals may continue to compete in the reproductive tract of the hens both by numerical advantage and by relative physiological virility. This problem is being directly attacked by one of us with attention to all the known variables (cf. Guhl, Warren, and Payne, 1944).

There can be no doubt but that social dominance among males, whether of *G. domesticus*, the sage grouse (Simon, 1940; Scott, 1942), or rhesus monkeys (Carpenter, 1942), allows the dominant animal an opportunity to sire more offspring. The relation between high social status and reproductive success of common hens is not so obvious. Sanctuary (1932) found that hens from the lower half of the peck-order lay fewer eggs than do their more aggressive flockmates. The high-ranking hens obtain more food and otherwise lead more secure lives. Opposed to these trends, there is the tendency for socially high-ranking hens to mate less frequently than those lower in the social scale. In our small flocks each hen mated frequently enough to insure fertile eggs and viable offspring. A preliminary report of a direct test (Guhl, Warren, and Payne, 1944) of the subject indicates that, despite the negative correlation with mating frequency, high-ranking hens, as well as cocks, produce more offspring than their low-ranking flockmates.

SUMMARY

1. Approximately 1,630 treadings of domestic fowl were observed and studied in relation to associated behavior and social status.

2. Statistically significant negative correlations were found between the social position of the hens and the frequencies at which they were courted by the cocks.

3. Although rarely statistically significant in any given flock, there was a consistent tendency toward a negative correlation between the frequency at which the hens were mated and their position in the social order that became statistically significant when results from different flocks were considered together.

4. In 4 out of 5 flocks the hens highest in the social order either failed to "invite" the cock to mate or crouched less frequently than most of their penmates. The consistent negative correlation between social rank and the frequency of giving the sexual crouch was suggested but not proved by our data.

5. There was a high correlation in all observed flocks between the number of times the individual hens were mated and their frequencies in giving "sex invitations" to the males.

6. There was no statistically significant relationship between the social status of the cock in his unisexual group and his sexual activities when placed alone in a flock of hens. However, when 4 cocks were introduced together into a relatively small but uncrowded pen containing hens, a form of suppression developed which practically inhibited mating behavior by some of the low-ranking cocks and demonstrated that the dominant males possess a greater freedom to mate and so sire more offspring than their socially inferior penmates.

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BISEXUAL MATING BEHAVIOR IN THE MALE RAT: EFFECTS OF CASTRATION AND HORMONE ADMINISTRATION[1]

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THE spontaneous execution of feminine mating behavior by male mammals is not common, and only two observers have reported this phenomenon in the case of the rat. Stone (1924) described two sexually active males of this species that displayed elements of the feminine copulatory pattern when they were mounted by other males, and Beach (1938) observed one comparable case. The three animals examined by these two experimenters had not been subjected to special experimental treatment, and the sexual behavior of the males was noteworthy in that they showed vigorous masculine mating responses when confronted with receptive females. The animals could not properly be termed "feminized," since their copulatory reactions were actually "bisexual," being governed, to a large extent, by

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the nature of the external stimulus situation.

It is the purpose of the present paper to report the discovery of one more male rat that showed male sexual behavior when placed with oestrous females and female mating reactions when mounted by other males. The effects of castration and of subsequent administration of androgen and of estrogen will be described, and the bearing of the results upon theories of the neurohormonal control of sexual activity will be discussed.

The rat with which this report is concerned was one of several chosen to serve as "indicator males"—animals used to test sexual receptivity in females. Males employed in this manner are selected on the basis of demonstrated sexual aggressiveness. To be useful they must exhibit persistent and vigorous copulatory behavior even though the female is resistant and attempts to elude their attentions. Indicator males customarily become highly active as a result of their brief contacts with one female after another, and under such conditions they frequently mount one another after the female has been removed from the testing cage. It was under these conditions that the experimental male here described was first observed to execute feminine mating behavior.

METHODS

As soon as the male's propensity for bisexual behavior was discovered, a program of testing was instigated to measure, as accurately and quantitatively as possible, the frequency and completeness of both masculine and feminine responses and to define the stimulus conditions under which each type of behavior was most likely to be elicited.

In all behavior tests the animals were placed in a circular metal cage, 34 inches in diameter. The experimental room was quiet throughout the observational period. The test period was variable in length, but usually ranged from 5 to 10 minutes for each set of conditions to be described. On some days two tests were conducted with an intervening rest period of about ½ hour.

Three test situations were devised. Masculine copulatory behavior was measured by placing the experimental male with a highly receptive oestrous female and recording the number of incompleted attempts to mate, the number of completed copulations (with intromission), and the number of ejaculations.

Tests for feminine behavior were conducted by introducing the experimental male into the testing cage with three or four other males known to show high sexual aggressiveness. It was found essential that the stimulus males be highly aroused before the experimental individual was introduced. The usual procedure was to allow these males to copulate several times with a receptive female who was removed before the males could ejaculate. The experimental male was then immediately placed in the testing cage. The experimental male was also put with oestrous females and permitted to mate a few times before being transferred to the test cage. It appeared that the feminine sexual responses of the male occurred most readily if he had been stimulated by preliminary contact with the receptive female.

In testing for feminine sexual reactions no attempt was made to hold the experimental conditions rigidly constant. On the contrary, test length was variable; the animals were handled a great deal; and the conditions were manipulated in any way that promised to increase the incidence of the behavior under scrutiny. For example, the experi-

mental male was repeatedly lifted from the cage and immediately reintroduced, because this treatment often stimulated the other males to renewed mounting activity. If sexual responses declined during the test, a receptive female was occasionally dropped into the cage and left just long enough to revive copulatory behavior on the part of the males. During the tests for feminine reactions records were kept showing the number of times the experimental male was mounted, the number of times he exhibited lordosis (concave arching of the back and elevation of the pelvic region), the number of ear-vibration reactions, and the number of hopping responses. These three behavioral items constitute the mating pattern of the receptive female.

In one more experimental situation which was employed, three or four stimulus males and one or two receptive females were placed in the test cage with the experimental animal. Under these conditions the male had opportunity to show masculine reactions in a highly competitive situation, since other males were constantly active and might preempt the services of the females unless the experimental animal was sufficiently vigorous and prompt in his behavior. At the same time, the generally high level of arousal engendered in all animals and the fact that more males than females were present led to frequent mounting attempts between males. Thus, the experimental rat was often mounted in sexual fashion within a second or two of the time that he had copulated in masculine fashion with the oestrous female.

Five tests, including these three conditions—male with receptive female, male with other males, and male with both males and females—were conducted at irregular intervals over a period of approximately 3 weeks. The experimental male was then castrated, and the testes and a section of one seminal vesicle were preserved for histological study.

The first postoperative test occurred 6 days after castration; and a second test, conducted 5 days later, was followed by intramuscular injection of 0.5 mg. of testosterone propionate.[2] An equal amount was injected each day thereafter; and tests were carried out after the male had received 1.0, 1.5, 4.5, and 6.0 mg. of the androgen.

A subsequent rest period of 6 weeks was allowed so that the effects of the injected hormone might be dissipated, and then testing was resumed. A series of five examinations occurred during the next 11 weeks. Administration of estrogen followed;[3] and 100 rat units (r.u.) were injected each day for a period of 5 days, and three tests were conducted during this time. The rat was observed after receiving a total of 200, 400, and 500 r.u. of the hormone.

A final test was conducted 19 days later, and in preparation for this examination the male was subjected to the standard hormone treatment regularly used in our laboratory to induce full sexual receptivity in spayed female rats. A single injection of 500 r.u. of estrogen was followed after 48 hours by the administration of 0.5 mg. of progesterone, and the test occurred 12 hours later.

RESULTS

Data included in Table 1 show the types of behavior elicited before and

[2] The hormone preparation employed was "Oreton" and consisted of testosterone propionate in oil at a concentration of 5 mg. per cubic centimeter. This material was generously supplied by Dr. Max Gilbert of Schering Corporation, Bloomfield, New Jersey.

[3] Dr. Max Gilbert also kindly provided a supply of "Progynon-B" (estradiol benzoate in oil, 1,000 r.u. per cubic centimeter).

after castration and hormone administration. Not all observation periods are represented; and since tests varied in duration, scores recorded on different days are not directly comparable. However, the figures presented do indicate accurately the trend and general magnitude of changes occurring in the experimental animal's behavior.

TABLE 1

EFFECTS OF CASTRATION FOLLOWED BY HORMONE ADMINISTRATION UPON FREQUENCY OF MASCULINE AND FEMININE MATING RESPONSES

(Test Length Variable)

Condition of Male	Normal			Castrated														
				Androgen-injected											Estrogen-injected			Estrogen- and Progesterone-injected
Day of experiment	1	13	17	23	28	35	37	42	44	90	104	170	171	174	176	179	182	201
Masculine responses:																		
Copulations	5	9	13	14	0	12	18	18	22	0	0	0	0	0	7	0	0	6
Ejaculations	2	3	4	4	0	0	2	5	2	0	0	0	0	0	0	0	0	0
Feminine responses:																		
Times mounted	13	13	19	20	25	26	45	58	90	19	9	8	3	0	19	14	10	25
Lordosis	11	12	12	0	0	0	11	44	60	0	0	0	0	0	9	13	10	21
Hopping	11	7	10	10	0	0	6	33	39	0	0	0	0	0	0	0	0	19
Ear-wiggling	9	2	7	0	0	0	0	0	7	0	0	0	0	0	0	0	0	16
Attacks	11	11	3	4	0	0	0	14	11	0	0	0	0	0	0	0	0	0

BEHAVIOR BEFORE CASTRATION

Masculine responses.—All indications based upon tests conducted before castration point to the conclusion that the experimental animal was an extremely vigorous and effective copulator. In the test situation which involved contact with the receptive female exclusively, the male initiated mating with little delay, executed very few incomplete attempts, and ejaculated after a surprisingly few completed copulations. The average male from our colony copulates approximately seven or eight times before ejaculating; and although individual differences are marked, it is rare that ejaculation occurs before the fourth or fifth contact with intromission. The male observed in the present experiment frequently ejaculated on the third copulation and occasionally did so as early as the second.

The testing technique used in this experiment differed in several respects from the techniques which we have employed in earlier investigations of masculine sexual behavior of the rat, and therefore a direct comparison of the performance of the male considered in this study with that of larger groups examined previously is impossible. However, the data presented in Table 1 establish, beyond question, the fact that this individual was at least as persistent and efficient as the general run of animals from our experimental stock.

Feminine responses.—When one male rat is mounted by another, the usual response is an immediate attempt to dis-

lodge the mounting animal and to repel any subsequent endeavors to mount. Most frequently the rat that is mounted twists sideways and pulls away, or rises on the hind legs and turns to face the other male, pushing him away by striking motions of the forepaws. In some cases, although not frequently, the male which is mounted merely "humps" his back and makes little effort to avoid contact.

The animal with which this report is concerned sometimes repelled other rats which tried to mount him. More frequently, however, he responded in a manner typical of the oestrous female. When approached by other males, the experimental rat darted away for a short distance and then crouched with the back somewhat depressed and the rear legs spread slightly. If the pursuing male mounted and clasped, the experimental animal exhibited pronounced lordosis and opisthotonus. Often the male executed rapid ear vibrations, characteristic of the highly receptive female. The completeness and intensity of the feminine responses varied from time to time; but in many tests the pattern was elicited so frequently and readily, and was performed with such vigor, that the total picture was momentarily indistinguishable from that presented by a female at the peak of receptivity.

In other reports (Beach, 1944) we have described quantitative measures of sexual receptivity in the female rat. Variability of observational techniques in the present study preclude any direct, quantitative comparison between the sexually receptive responses of the experimental male and those of the normal oestrous female. Nevertheless, values shown in Table 1 demonstrate that, although it was highly variable, the feminine performance of the experimental male occurred very frequently and often included all of the separate elements normally integrated into the mating response of the female in heat.

Bisexual responses in a competitive situation.—Observation of the sexual reactions of the experimental male during test periods in which both male and female stimulus animals were available revealed several significant points. In the first place, the male's masculine responses were not at all inhibited in a competitive situation. On the contrary, the experimental individual customarily dominated the mating situation, usually being the first male to copulate with a receptive female and invariably reaching the point of ejaculation before any of the other males.

Secondly, it was under this set of testing conditions that the male's feminine responses were most frequent and complete. The observer gained the impression that repeated contacts with the receptive female threw the experimental male into a state of high sexual excitement and that under such conditions the mounting activities of stimulus males were most likely to elicit prompt and intense oestrous responses.

A third and extremely interesting aspect of the animal's behavior in a competitive situation revealed itself in the appearance and progressive intensification of the aggressive reactions which are described below.

Aggressive behavior.—At the beginning of the testing schedule very little fighting occurred; but in each succeeding test prior to castration the amount of such behavior on the part of the experimental animal increased. The attacks of the experimental male were confined almost exclusively to other males. Of a total of more than fifty attacks, only one was directed toward the receptive female; and

in this instance the female bit the male repeatedly before he retaliated. In one instance a dioestrous female was accidentally included with the stimulus animals, and this individual was attacked once.

Aggression seemed to be elicited chiefly by one of two stimulus conditions. Very frequently the experimental male displayed feminine sexual behavior so complete that it stimulated another male to mount and attempt copulation. The experimental male completed the feminine pattern by assuming the lordosis position and then, after the stimulus animal dismounted, turned and launched a vicious attack. A large proportion of the experimental male's aggressive reactions thus came as a direct result of mounting activity which his feminine behavior had induced.

Many other attacks followed immediately after an ejaculation by the experimental male at a time when no mounting activity had been shown by the stimulus males. In the competitive situation the male frequently copulated two or three times with the oestrous female, ejaculated, and then turned immediately to attack the nearest stimulus male.

The viciousness of such behavior on the part of the experimental animal had a markedly depressing effect upon the sexual activity of stimulus males. They soon ceased to mount the experimental male and showed strong avoidance reactions whenever he was placed in the testing cage. In a fairly short time the competitive testing situation took on the aspect of a solo demonstration by the experimental animal, for, so long as he was present, other males failed to make any attempt to copulate with the receptive female. To counteract this unforeseen development, stimulus males were injected with large amounts of testosterone propionate, with the result that their sexual behavior increased in intensity and persistence; and they once more showed willingness to mount the experimental male despite his punitive reciprocities.

BEHAVIOR AFTER CASTRATION

The first postoperative test, conducted 6 days after castration (day 23, Table 1), revealed several pronounced changes in the sexual performance of the experimental animal. Masculine copulatory responses were not significantly altered. Mating activity began with little delay, incomplete copulations were infrequent, and ejaculations occurred after an average of approximately three intromissions.

Feminine mating reactions were nearly completely abolished. Although the male was mounted a total of twenty times, he showed no lordosis responses and no ear vibration. Some hopping behavior occurred. In a 60-minute test the experimental male attacked stimulus males a total of four times. The attacks occurred all at once, followed immediately an ejaculation by the experimental male, and were directed toward other males which had attempted to mount him.

With the exception of this brief flurry of aggressive behavior, the response of the experimental animal toward other males showed marked change from preoperative standards. He frequently repelled the mounting attempts of other males by raising the rear quarters and kicking backward with one hind leg in a manner typical of the dioestrous female. He retreated from, and made every effort to avoid contact with, other males; and when their attentions became more pressing, he tried several times to jump out of the testing cage. The mere approach of a stimulus male was sufficient to evoke loud squeals from the experimental animal.

A second test, conducted 11 days after castration, showed further change in the male's sexual behavior (day 28, Table 1). Masculine copulatory reactions had been drastically reduced. In a 20-minute period with three very receptive females the male's mating responses were confined to two incomplete attempts to copulate; and in the competitive situation he showed no reaction whatsoever to the stimulus females.[4] During 30 minutes with stimulus males the experimental animal was mounted a total of twenty-five times but showed no lordosis, hopping, or ear-wiggling. No attacks occurred; and the male's response to the approaches of stimulus animals consisted exclusively of attempts to evade contact and to escape from the testing cage.

BEHAVIOR AFTER ANDROGEN ADMINISTRATION

Twenty-four hours after receiving 0.5 mg. of testosterone propionate the experimental male was tested with two receptive females for a period of 20 minutes, during which time he showed fifty-six incomplete attempts to mate and twelve copulations. No ejaculations occurred. When placed with males and females simultaneously, the experimental animal made no response to the females but was mounted twenty-six times by other males and failed to exhibit any feminine mating reactions (day 35, Table 1).

Tested 24 hours after the third daily injection of 0.5 mg. of androgen, the male demonstrated a return of ejaculatory power and willingness to copulate with the receptive female in the competitive test situation. During the same period he was mounted forty-five times and showed eleven lordosis responses and six hopping reactions. The rat's masculine responses were not as persistent as they had been before operation; and lengthy delays usually intervened between successive copulations. Feminine reactions were sluggishly and weakly performed, but resistance to mounting attempts by other males was definitely decreased.

The remainder of the results pertaining to performance under the influence of injected androgen can be seen in data embodied in Table 1. It is apparent that by the time a total of 4.5 mg. of androgen had been administered, the experimental animal's reactions were nearly comparable to those seen in preoperative tests (day 42, Table 1). Copulation, ejaculation, lordosis, and hopping occurred at frequencies comparing favorably with those seen in initial observational periods. Aggressive behavior had returned, and the majority of attacks once more occurred shortly after ejaculation by the experimental male. Administration of two additional 0.5-mg. injections resulted in an increased frequency of feminine responses but had no apparent effect upon masculine behavior (day 44, Table 1).

Tests conducted from 6 to 17 weeks after androgen administration revealed that the effects of the injected hormone wore off rapidly, and the sexual activity of the experimental male returned to the castrational level (days 90–174, Table 1). In a series of five tests conducted over a 3-month period the experimental male showed occasional, incomplete masculine reactions, no semblance of feminine

[4] Although some male rats cease to show any mating behavior within a few days after castration, the majority of individuals tend to exhibit at least incomplete copulatory attempts for a considerably longer postoperative interval (Stone, 1927). It is quite likely that under more exhaustive testing techniques the male in the present experiment would have demonstrated sexual activity for a longer time than these results indicate.

behavior, and total loss of all aggressive tendencies.

One important feature of this series of tests lay in the observation that stimulus males showed very little tendency to mount the experimental animal after he had ceased to display feminine reactions. This is peculiar, because in the same tests the stimulus males repeatedly mounted one another. Two explanations present themselves. Stimulus males were less vigorous in their attempts to repel mounting by other males than was the experimental animal. Although, in most instances, they dislodged mounting animals promptly, there was no consistent attempt to prevent the occurrence of mounting. The castrated male, in contrast, raised his hind quarters and kicked backward vigorously in an effective attempt to ward off other males, and thus often prevented any mounting whatsoever. Another factor which undoubtedly contributed to the decrease in the frequency with which stimulus animals mounted the experimental male lay in his relative inactivity. When placed in the testing cage with other males, this animal typically moved to the far wall of the cage and huddled motionless for the duration of the test. If he was lifted and dropped in the center of the floor, he immediately retreated to his former position. Stimulus males, on the contrary, were highly aroused, constantly active, and therefore frequently elicited sexual investigation and copulatory attempts on the part of other males.

BEHAVIOR AFTER ESTROGEN ADMINISTRATION

Approximately 3 months after the last injection of androgen the administration of estrogenic hormone was begun. One hundred rat units of estradiol benzoate were injected intramuscularly every day for a period of 5 days; and tests were conducted 24 hours after the second, fourth, and fifth injections.

After receiving 200 r.u. of the hormone the male displayed a partial revival of both masculine and feminine mating responses (day 176, Table 1). When placed with receptive females for 12 minutes, the experimental animal made nine incomplete attempts to mate and copulated seven times, but failed to ejaculate. No masculine reactions occurred in the competitive test situation; but the male was mounted nineteen times and exhibited lordosis in nine instances. Lordosis was sluggishly performed, and there was still a good deal of resistance to the mounting attempts of stimulus males. Hopping and ear-wiggling did not occur.

After receiving a total of 400 r.u. of estrogen the experimental male showed significant increase in the readiness to display lordosis when mounted by other males (day 179, Table 1). No other elements in the feminine copulatory pattern appeared; but avoidance reactions to the masculine advances of stimulus males had been eliminated. At the same time, all traces of masculine copulatory behavior disappeared. The male showed no tendency to investigate or mate with receptive females.

Administration of an additional 100 r.u. of the hormone brought about no perceptible change in sexual activity (day 182, Table 1).

In describing his performance under the influence of injected estrogen it should be added that the experimental animal was definitely in poor health during this part of the experiment. Advanced respiratory infection was evident, and throughout the majority of tests the male was totally inactive. When transferred from one part of the cage to another, he usually remained motionless

wherever the experimenter had placed him. Some difficulty was experienced in inducing the stimulus males to mount the inactive animal; but whenever they did so, the lordosis response was prompt and complete.

The administration of estrogen, followed by progesterone, resulted in an increase in the completeness of the feminine sexual pattern and partial revival of the masculine mating reactions (day 201, Table 1). Lordosis occurred in response to twenty-one out of twenty-five mounting attempts by stimulus males, and hopping and ear-wiggling were shown repeatedly. The experimental male made twenty-four incomplete attempts to copulate with receptive females, and six completed copulations with intromission were recorded. Penile erection was observed, but ejaculation did not take place.

HISTOLOGICAL EXAMINATION

Upon removal, the testes of the experimental animal proved somewhat smaller than average size for males of his age and body weight, but measurements fell well within the range of normal variation. The left testis was 14 × 10 mm. in largest dimensions, and weighed 0.77 gm. The right was 16 × 11 mm. and weighed 0.94 gm.

Histological section revealed normal tubules with active spermatogenesis. Interstitial tissue was normal in amount and in histological appearance. The epididymis was full of sperms and exhibited no atypical features.

A sample section of the seminal vesicle bore high epithelium with granular inclusions and clear areas in the cytoplasm, indicating normal secretory function.

All histological data indicate that the gametogenic and secretory activities of the testes in the experimental male were normal.

DISCUSSION

Three independent variables contribute to the control of mating behavior in male vertebrates, and any adequate interpretation of sexual arousal and copulatory responses must take all three into account. One variable, which is often mistakenly assumed to be a constant, involves the character and intensity of the external stimulus. The execution of normal masculine copulatory performance by the male demands the presence and co-operation of the receptive female, and the behavior of individual females is sufficiently different to affect the completeness and intensity of the male's response. A second variable rests upon the organization of the central nervous system and the muscular effector units. No matter how adequate the external stimulus may be, it is powerless to evoke behavioral responses unless their mediation is provided for in the neuromuscular constitution of the animal subjected to stimulation. A third variable is related to the effects of endocrine secretions, particularly those of gonadal origin. Findings of numerous experiments have made plain that normal function of the nervous elements involved in sexual behavior depends upon the presence of sex hormones.

NERVOUS MECHANISMS

We have previously suggested (Beach, 1942*a*) that the masculine sexual behavior of the male rat is mediated by innately determined central nervous mechaisms, and it is our belief that the functional organization of these neural relationships is one fundamental feature of the genetically controlled sexual constitution. The preorganized neuromuscular apparatus responsible for overt mating responses may logically be regarded as an essential unit in the reproductive system, comparable in many respects to the

accessory sexual structures or to certain secondary sex characters. Developmental differentiation of the mechanisms for courtship and copulatory behavior is, in all probability, controlled and directed by the same genic forces which influence similar processes in other reproductive structures.

There are various lines of evidence indicating that the genetic endowment of males of at least some species includes neuromuscular mechanisms capable of implementing the execution of feminine sexual behavior. This hypothesis, which in effect supposes a dual or bisexual arrangement within the central nervous system, has been proposed by several writers, including Sand (1919–20), Ball (1939), Beach (1941), and Engel (1942). Observations such as those of the present investigation definitely indicate the existence of feminine mechanisms in at least some untreated males. In addition, there have been reported various successful attempts to evoke female mating responses in male animals after experimental alteration of the endocrine balance. Such experimentally induced effects, which have been produced in male birds (Crew, 1927; Domm, 1939), reptiles (Noble and Greenberg, 1941), and mammals (Steinach, 1911; Moore, 1919; Ball, 1939; Beach 1942*b*; Engel, 1942) point to the conclusion that all males possess the essential nervous organization for feminine behavior and that under appropriate hormonal conditioning the overt female responses can be elicited.

If we postulate a bisexual arrangement of the neural substrate involved in mating activity, we are faced with the necessity of explaining why the majority of males exhibit the masculine sexual pattern exclusively; and why, at the same time, there occur occasional individuals which spontaneously display feminine, as well as masculine, reactions. It may be suggested that the same factors which affect all other morphological manifestations of sex also exert a determining influence upon the relative sensitivity of the behavioral mechanisms and that in most males the nervous circuits for feminine behavior are comparatively unresponsive to stimulation, while the mechanisms for masculine sexual performance are highly reactive. Under such conditions the exceptional male which shows female responses can be accounted for by assuming the existence of an atypically high degree of sensitivity on the part of the nervous elements in which feminine behavior is organized. The assumption of this extreme type of individual variation is quite in accord with the genic balance theory of sex determination, for, as Willier (1939) has pointed out, the theory provides for the fact that a tendency to develop feminine characters will be stronger in some male embryos than in others.

One final point which should be included in our discussion of the nervous mechanisms involved in bisexual behavior is the question of a general level of sexual arousal. It is extremely significant that the few male rats which have been observed to display oestrous responses have also proved to be exceptionally vigorous copulators when placed with receptive females. They have been, in other words, more than normally responsive to any sort of sexual stimulation. Furthermore, the feminine behavior of these individuals has appeared most frequently and in greatest detail under conditions calculated to engender a state of intense sexual excitement—namely, at times when the males had recently copulated in masculine fashion with the oestrous female and when ejaculation was imminent.

These observations lead us to believe that the execution of feminine mating behavior by untreated males demands a very high degree of sexual arousal, and evidence which supports this belief is found in the report that male rats which do not spontaneously exhibit female responses may do so if injected with very large amounts of androgen, which greatly increase sexual excitability and intensify masculine mating tendencies (Beach, 1941).

The foregoing analysis provides an interpretation of the bisexual behavior presently described. It is implied that males which exhibit feminine sexual responses without special treatment do so because they deviate from the average in two ways. The mechanisms for female behavior, which are present in all males, are unusually responsive to stimulation in the exceptional individuals under consideration. In addition, the capacity for sexual arousal is extremely high. It is to be understood, of course, that external stimuli appropriate to the elicitation of feminine performance must be frequently and forcefully applied. It should be noted that this theoretical point of view does not assume any abnormality of endocrine function in the males which display female behavior, and it can be shown that any such assumption is superfluous.

HORMONES

We have previously referred to experiments in which male animals were found to exhibit feminine sexual reactions after being implanted with ovarian tissue or injected with estrogenic hormones. Originally such results were regarded as examples of "behavioral sex reversal," but more recent studies cast some doubt on this interpretation. It seems more logical to postulate that the experimental procedures resulted in the sensitization of previously refractory behavioral mechanisms. To explain this viewpoint, it is necessary to devote some attention to the role of gonadal hormones in sexual activity.

Androgen and estrogen exert many diverse effects upon the organism, but a large measure of their influence over behavior is thought to reside in their capacity to affect the function of central nervous factors responsible for the organization of certain overt responses (Rioch, 1938; Bard, 1939; Young and Rundlett, 1939; Lashley, 1938). The sex hormones, in so far as they enter the behavioral picture, are best regarded, not as stimuli or as organizing agents, but as chemical sensitizers which alter the stimulability of critical mechanisms within the central nervous system (Beach, 1945). Thus, one function of androgen is to increase responsiveness to stimulation in the neural circuits controlling masculine mating behavior, and one effect of estrogen is to bring about comparable changes in the central mechanisms for feminine sexual reactions.

This concept obviates any necessity for the postulation of an "antagonism" between the effects of testicular and ovarian secretions. We suggest, on the contrary, an affinity between androgen and the mechanisms for masculine responses, and a similar relationship between estrogen and feminine mechanisms. It may be further stipulated that, under ordinary conditions, the sensitizing function of these hormones is so important that in their absence the reactivity of the affiliated nervous mechanisms is sufficiently reduced as to preclude the occurrence of mating behavior even though the appropriate external stimuli may be applied.

This type of interpretation can be employed to explain earlier findings which have been regarded as proof of sex reversal or of hormone antagonism. Steinach

(1911) and Moore (1919) have reported that male rats which were castrated and implanted with ovarian tissue ceased to display masculine mating responses and began to exhibit the feminine copulatory pattern. According to the foregoing line of reasoning, the disappearance of male behavior should be referred to loss of testicular androgen and consequent decrease in the sensitivity to stimulation of the central nervous mechanisms mediating masculine copulatory performance. At the same time, estrogen secreted by the implanted ovary would increase the responsiveness of neural circuits controlling feminine reactions, and with appropriate external stimulation such responses would be executed. No inhibitory effects need be assumed. One behavioral system has been deprived of its normal hormonal facilitation, and another has been supplied with such aid, which was previously lacking.

There are, of course, several reports revealing a decrease in the sexual activity of male animals following the administration of estrogen. For example, Mulligan (1944) has recently noted that male dogs treated with estrogen cease to mate with the oestrous bitch. Even in such cases there is no necessity to assume that the exogenous hormone has exerted an antagonistic or inhibiting effect upon masculine sexual tendencies. A more logical interpretation would seem to be that the injected steroid depressed the gonadotrophic function of the anterior pituitary and thus indirectly interfered with the secretion of endogenous androgen, which is essential to full sensitivity in the nervous mechanisms for the behavior in question.

Far from inhibiting the appearance of male behavior, estrogen may increase the reactivity of central nervous mechanisms for masculine sexual performance. The effect is much less pronounced than that produced by androgen, but its existence is strongly indicated by results of several experiments.

In the present report it was shown that injections of estrogen not only revived feminine mating responses but also effected partial restoration of masculine copulatory behavior. Ball (1937) has noted the recurrence of copulation and ejaculation in castrated male rats treated with estrogen, and confirmatory results are reported by Beach (1942*c*). According to Davis and Domm (1943), capons exhibit masculine copulatory behavior following estrogen administration.

SUMMARY

One male rat which was an unusually vigorous copulator when placed with females in heat was observed to display all elements of the feminine mating pattern when mounted in sexual fashion by other males. The experimental animal was highly aggressive and frequently attacked stimulus males after they had mounted him and elicited feminine reactions.

Castration was followed by the immediate loss of feminine mating reactions, and masculine copulatory behavior and aggressiveness disappeared somewhat later.

Injections of testosterone propionate restored copulation, the power of ejaculation, and aggressive reactions and also revived all aspects of the feminine mating pattern.

Injections of estrogen and progesterone evoked male copulatory behavior without ejaculation and called forth all of the oestrous responses.

The bearing of the foregoing observations upon general concepts of the neurohormonal basis for sexual behavior is discussed.

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INFLUENCE OF THE STIMULI PROVIDED BY THE MALE CICHLID FISH, TILAPIA MACROCEPHALA, ON THE SPAWNING FREQUENCY OF THE FEMALE[1]

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THE influence of sexual exteroceptive factors on the ovulatory cycle has been established in a number of mammals and birds. The rabbit, cat, ferret, and ground squirrel usually ovulate only after coitus or comparable stimulation, while the pigeon and ringdove are outstanding examples of birds requiring sexual stimulation (courting and copulation) before they will ovulate.

In two recent reviews of this subject (Hartman, 1939; Marshall, 1942) this concept of the ovulatory cycle's being influenced by sexual stimuli arising from behavioral relations between the male and female (or sometimes between two females) has been extended to include the lower vertebrates. However, the evidence presented is rather meager. Regarding the teleosts, two publications are cited. First, in a paper by Hamlyn-Harris (1931), who studied the Australian trout gudgeon, *Mogurnda* (*Mogurnda*) *adspersus*, the statement is made that "oviposition does not take place, however, unless the male is sufficiently energetic in his attentions, which take the form of a constant display of nuptial colouring and, in addition, a tendency to 'knead' the body of the female with his mouth." In the second reference Hobbs (1937), who investigated the New Zealand quinnat salmon and brown trout, states (p. 40) that "the presence of a male is not necessary to stimulate the female into preparing a redd, but is necessary to excite oviposition." Unfortunately, neither of these conclusions is supported by experimental data or extensive observations. On the other hand, in the voluminous fish literature one can find statements such as those of Rutter (1902) and of Schreitmuller (1937) suggesting that the presence of the male is not necessary for ovulation and oviposition.

Fish of the family cichlidae are noted for their elaborate courtship patterns; and one can easily conceive that, prior to spawning, the male and female are mutually stimulating each other. Hence it was considered appropriate to examine this question, using as experimental material a species of fish belonging to this family. The purpose of this investigation was to determine whether the male stimulates the female to ovulate and oviposit, and, if so, by what sensory modalities the stimuli are transmitted.

MATERIALS AND METHODS

The species used was the African mouth-breeding cichlid, *Tilapia macrocephala*. The elaborate courtship and

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spawning behavior of this fish will be outlined here in the briefest possible manner, since this subject will appear in another report (Aronson, in preparation). Prior to the spawning the male and female build a shallow depression, or nest, by scooping out the gravel on the bottom of the tank with their mouths; and while this nest building is in progress, various additional phases of courtship and oviposition behavior are displayed. These include (1) nipping; (2) a marked expansion of the throat and gill arches (throat puff); (3) nodding the head; (4) tail slapping; (5) vibration of the body musculature (body quiver); (6) swimming slowly over the nest and rubbing the genital tube on the substratum (passing nest); (7) vibration of the body musculature while passing nest (spawning quiver); and (8) deposition of eggs or sperm during a spawning quiver. The eggs are generally picked up by the male, but sometimes by the female, and are retained in the parent's mouth for 2 or 3 weeks, during which time they hatch and the young develop.

The following six experimental conditions were established (Fig. 1): (1) male and female together in a 54-liter aquarium, 60 × 30 × 30 cm.; (2) male and female in a 54-liter aquarium, separated from each other by a transparent glass fence which divided the aquarium into two equal halves (this fence prevented the fish from coming in contact with each other but allowed the water to pass freely from one side of the tank to the other); (3) male and female in a 54-liter aquarium separated by a transparent glass partition sealed into the tank with pitch; (4) male and female in a 54-liter aquarium separated by an opaque glass partition sealed into the tank; (5) male and female in separate 26-liter aquaria, 35 × 30 × 25 cm., placed side by side, in which the adjacent walls were transparent; and (6) male and female in separate 26-liter aquaria, the adjacent walls being opaque.

The first experimental condition represented the control. The second condition eliminated stimulation by physical contact and possibly dampened the transmission of vibratory stimuli through the water. The transparent partition eliminated contact and chemical stimulation and further reduced the possibility of vibratory stimuli. With the opaque partition optic stimuli were also eliminated. The fifth condition allowed visual stimulation but completely prevented contact, chemical, and vibratory transmission. In the sixth and last situation the female was completely isolated from the male.

These six experimental situations just described will be referred to henceforth as a "group." Each of the experimental conditions was repeated nine times. Thus there were nine groups. The first four groups were placed in room A of a large greenhouse, and groups 5–9 were placed in adjacent room B. The temperature in the greenhouse was maintained at approximately 27° C. at all times. The tanks were so arranged that the physical conditions, particularly illumination and nearness to radiators and windows, were as nearly alike as possible for every pair in a given group; but because of limitations of the space allotted for the experiment, variation in these conditions among the several groups could not be avoided.

The outside walls of all the aquaria were covered with a heavy coat of pale-blue paint except the side walls of certain of the adjacent aquaria mentioned above. Inspection holes, covered with cardboard flaps, were placed in the front walls of all aquaria. The height of the

water was adjusted in all the tanks so that the 26-liter tanks and partitioned halves of the 54-liter tanks contained roughly 18 liters, while 36 liters of water were placed in the control aquaria. Each tank or partitioned half was supplied with an aerator, a few leaves of the water fern *Ceratopteris*, and some snails. A foliage was removed from the tanks approximately once a month.

Young adults were distinguished by the appearance at sexual maturity of sexually dimorphic color patterns of the opercula. The fish were paired at random and placed in 54-liter preliminary spawning tanks. On the day they spawned, as

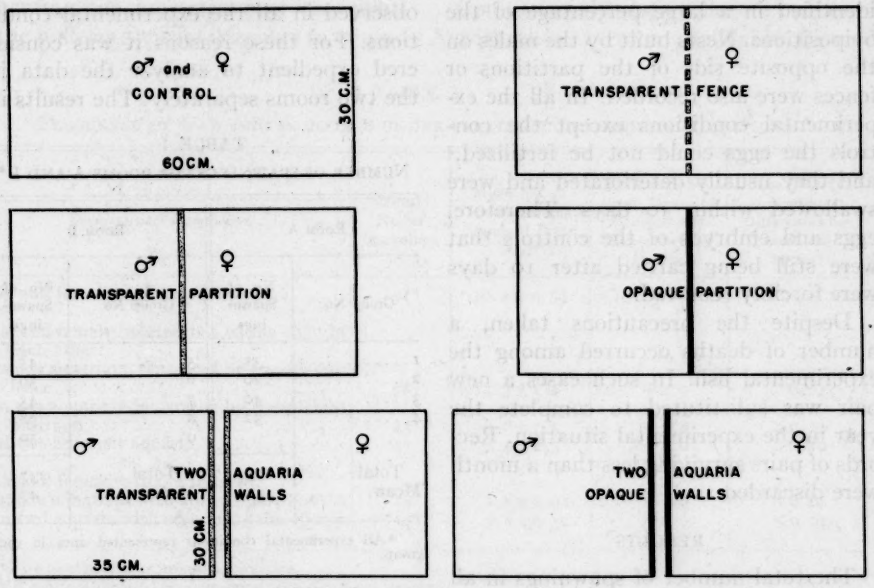

FIG. 1.—Diagram showing the arrangement of the tanks, partitions, and fence in the six experimental situations.

charcoal aquarium filter was placed in one partitioned half of each 54-liter tank and in alternate 26-liter tanks for a period of 2 weeks, after which the filter was cleaned and shifted to the opposite half or adjacent tank. To each 26-liter aquarium and to each half of the 54-liter containers 100 gm. of Louisiana rock salt were added to improve the health of the fish. Additional floating plants were placed on one side of each control tank, to afford hide-outs in the event of extreme pecking or fighting. Excessive

determined by the presence of eggs in the nest or in the mouth of the male or female, the pair was removed from the preliminary tank, weighed, the eggs were forcibly ejected from their mouths, and the pair was placed in one of the experimental situations described above. It generally took from 1 to 3 weeks to select a group of six pairs, but 7 months elapsed between the establishment of the first and last groups.

All experimental tanks were maintained for 1 year from the date of the

preliminary spawning, during which time they were inspected twice daily except Sundays (between 9:00 A.M. and 11:00 A.M. and again between 3:00 P.M. and 5:00 P.M.) for the presence of eggs which indicated that a spawning had occurred.[2] In addition to the record of the spawning, a notation was made of the location of the nests, which could be identified in a large percentage of the ovipositions. Nests built by the males on the opposite side of the partitions or fences were also recorded. In all the experimental conditions except the controls the eggs could not be fertilized,[3] and they usually deteriorated and were swallowed within 10 days. Therefore, eggs and embryos of the controls that were still being carried after 10 days were forcibly removed.

Despite the precautions taken, a number of deaths occurred among the experimental fish. In such cases a new pair was substituted to complete the year in the experimental situation. Records of pairs surviving less than a month were discarded.

RESULTS

The total number of spawnings in all the groups are listed in Table 1. It is seen that, despite our attempts to keep the conditions equal in the two rooms, there were considerably more egg-layings in the second room. Moreover, the variability of the data was much more pronounced in room A. The number of control spawnings, for example, varied in room A from one egg-laying in group 4 to nine in group 3; while in room B the minimum counted was nine spawnings in groups 6 and 7 and the maximum was twelve in group 5. A similar difference in the variability of the number of spawnings between the two rooms was observed in all the experimental conditions. For these reasons it was considered expedient to analyze the data in the two rooms separately. The results in room B will be presented in detail and will be compared in a general way with the data obtained in room A.

TABLE 1

NUMBER OF SPAWNINGS FOR ROOMS A AND B*

Room A		Room B	
Group No.	No. of Spawnings	Group No.	No. of Spawnings
1	31	5	45
2	20	6	39
3	40	7	45
4	32	8	58
		9	50
Total	123	Total	237
Mean	31	Mean	47

* All experimental conditions represented once in each group.

The group distribution of the 237 spawnings which were recorded during the year in room B (groups 5–9, incl.) is summarized in Table 2. It is seen that in the two experimental situations where the female was visually isolated from the male, there were considerably fewer ovipositions, as compared with the controls. Moreover, there was only a slight difference between the frequency of spawning of the females visually isolated by the opaque partition and those completely isolated in separate aquaria. This indicates that any vibratory stimuli

[2] It has been observed elsewhere that a small percentage of males and females will swallow their eggs shortly after picking them up. However, there was no indication that this tendency varied in the different experimental situations except that it occurs somewhat less frequently in the males (controls). In the data which follow, the number of spawnings recorded might be slightly smaller than the actual number occurring.

[3] The possibility was considered that males might be able to fertilize eggs through the glass fence; but in 102 such spawnings, no developing embryos were recovered.

that the males might have emitted, such as by tapping against the partition, were not effectual in substantially increasing the spawning activities of the female.

The females separated from the males by transparent glass partitions and those in separate tanks with transparent adjacent walls spawned about the same number of times as the controls, thus indicating the importance of the sight of the male as a visual stimulus to ovulation and oviposition. Again, no significant difference was noted in the frequency of spawning of those females separated from the males by glass partitions and those in adjacent aquaria. This demonstrates again that vibratory stimuli were not involved.

The females separated from the males by the transparent fence spawned somewhat more frequently than the controls; but, owing to the small number of cases, it is not clear whether this difference is statistically reliable. However, a slightly larger difference was found between the mean number of egg-layings of the females separated from the males by the glass fence and that of those separated by the glass partition. If real differences do exist, they can be attributed to either or both of two factors, namely, a chemical influence transmitted by the circulating water and vibratory stimuli conducted by the water through the slots in the fence.

Comparing the data collected in room A with those in room B (Table 3), we find the results to be approximately the same. However, it was not possible to demonstrate statistically the reliability of the differences between the means of the control and experimental situations in room B because of the more pronounced variability. The females separated from the males by the glass fence spawned somewhat more frequently than the controls. Similarly, the females in the aquaria with the transparent partition and those in the separate tanks with transparent adjacent walls spawned just about as often as the controls. Finally, in the two experimental situations

TABLE 2

FREQUENCY OF SPAWNING IN ROOM B UNDER VARYING EXPERIMENTAL CONDITIONS

Experimental Conditions	Total No. of Spawnings	Mean No. of Spawnings and Standard Error (σ_M)*	Difference between Means of Controls and Experimental Groups	Probability (P) of Significant Differences between the Means†
Male and female together (control)	52	10.4±0.59	.....	.....
Male and female isolated but within sight of each other:				
In one aquarium, separated by transparent fence	72	14.3±1.4	+4.0	0.045
In one aquarium, separated by transparent partition	43	8.6±1.3	−1.8	>0.10
In two adjacent aquaria	37	7.4±2.3	−3.0	>0.10
Male and female visually isolated:				
In one aquarium with opaque partition	14	2.8±0.91	−7.6	<0.01
In two aquaria with adjacent walls opaque	19	3.8±0.59	−6.6	<0.01

* Five pairs in each experimental condition.

† $P < 0.01$ is almost always significant; $P > 0.05$ is not significant.

where the females were visually isolated from the males, the frequency of spawning was considerably less than the controls.

An analysis of the intervals between successive spawnings throws some light upon the manner in which visual isolation affected the ovarian cycle of the females. If the distribution of the interspawning intervals for all the control pairs are plotted (Fig. 2, *A*), it is seen that 67.1 per cent of all the intervals fell between 8 and 28 days, with a mode of 15 days. The remaining intervals ranged from 29 days upward to almost a year. By selecting 28 days as an arbitrary dividing line, ovarian cycles of 28 days or less may be referred to as "short cycles," while the intervals over 28 days may be called "long cycles." The behavior of a typical control pair, illustrated in Figure 3, shows that a series of short cycles is followed by one or more long cycles. This type of ovarian activity is characteristic of a number of cichlid species under relatively uniform conditions of captivity. Breder (1934) noted that the short cycles of *Aequidens latifrons* were 25 days in length and were sufficiently regular to permit the anticipation of the spawning dates with a fair degree of accuracy.

Comparing the ovarian cycles of the control females with those of females in the three experimental situations wherein the male was visible (Fig. 2, *B*, *C*, *D*), it can be observed that the distributions of the interspawning intervals in these cases are essentially the same as the controls. The percentage of short cycles varied from 45.9 to 70.6, and the mode of the short cycle varied from 15 to 18 days. The ovarian activity of these females was also characterized by a series of short cycles followed by one or more long cycles.

In the two experimental situations wherein the females were visually isolated from the males, the ovarian activity was considerably altered. Only about 20 per cent of the cycles were of the short type (Fig. 2, *E* and *F*), and ten of the seventeen females under consideration exhibited only long cycles. It thus appears that the short ovarian cycle is dependent, to a great extent, on the ability of the female to see a male.

Prior to the experiment the females

TABLE 3

COMPARISON OF THE FREQUENCY OF SPAWNING UNDER VARYING EXPERIMENTAL CONDITIONS IN ROOMS A AND B

Experimental Conditions	Mean No. of Spawnings*	
	Room A	Room B
Male and female in a single aquarium separated by a transparent fence	7.5	14.4
Male and female together in a single aquarium (control)	6.0	10.3
Male and female in a single aquarium separated by a transparent partition	6.5	8.6
Male and female in separate aquaria; adjacent walls transparent	5.5	7.4
Male and female in a single aquarium separated by an opaque partition	3.0	2.8
Male and female in separate aquaria; adjacent walls opaque	2.3	3.8

* Four pairs in each experimental condition in room A; and five in room B.

were kept with the males in large stock tanks, and each female spawned with a male before being placed in the experimental aquarium. It is possible that in the case of the visually isolated females the effect of the stimulation by the male during the pretest contact might have

Under normal conditions of captivity most of the nest-building is done by the females (Aronson, in preparation). Sometimes, however, the male will assist the female in the construction of a nest. When a male and female are separated by a glass partition, they sometimes

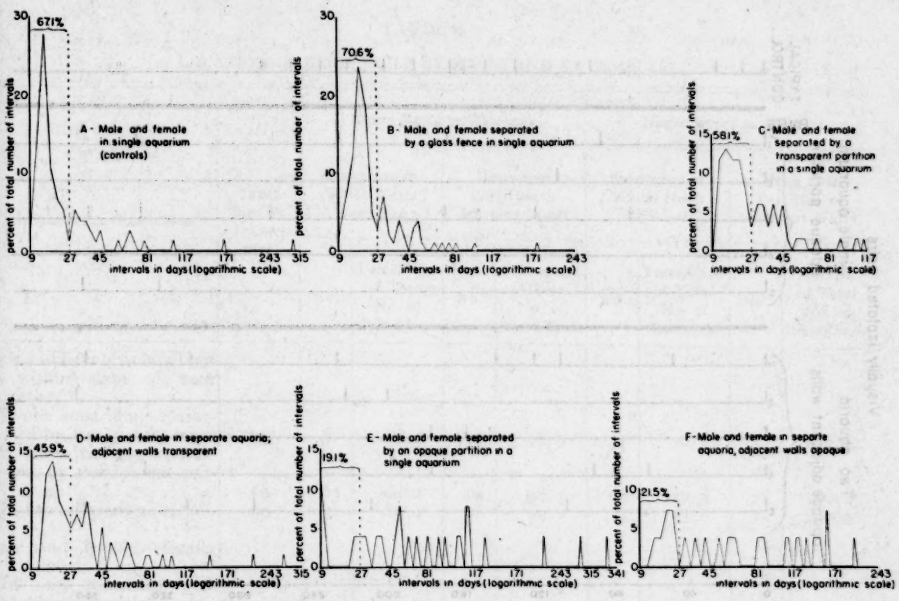
671%
A - Male and female in single aquarium (controls)
706%
B - Male and female separated by a glass fence in single aquarium
581%
C - Male and female separated by a transparent partition in a single aquarium
459%
D - Male and female in separate aquaria; adjacent walls transparent
191%
E - Male and female separated by an opaque partition in a single aquarium
215%
F - Male and female in separte aquaria, adjacent walls opaque
percent of total number of intervals
intervals in days (logarithmic scale)

FIG. 2.—Graphs illustrating distribution of interspawning intervals in the six experimental conditions. The intervals in days (abscissa) are plotted on a logarithmic scale. The broken lines represent the arbitrary division into short and long cycles. The percentage of short cycles is indicated in each graph. The peak at the extreme left of graph *E* represents three successive 8-day cycles for a single female. In the 360 intervals recorded these were the only cycles of less than 9 days. This peak is probably atypical.

persisted during a considerable part of the isolation period, thus accounting for the spawnings of these females. If such were the case, it would be expected that the spawnings of these females would be grouped toward the beginning of the isolation period. However, this was not the case. As illustrated in Figure 3, the egg-layings of the visually isolated females were distributed more or less equally throughout the experimental period.

build nests simultaneously, directly opposite each other on both sides of the partition. Nest-building activity on either side of a transparent partition has also been noted in other cichlid fish by Breder (1934) and by Noble and Curtis (1939). In the present experiment nests could often be recognized long after the eggs had been picked up, and wherever possible the locations of the nests in relation to the partitions were recorded.

Nests built by the females were identified in 85 to 100 per cent of the spawnings (Table 4). In the three experimental situations where the males were visible to the females, almost all the females' nests were located close to the partitions (within 5 cm.). On only five occasions were the nests constructed in other parts of the aquaria. When the females could not see the males, only a small number of nests were within 5 cm. of the opaque partitions or opaque adjacent walls. The strip 5 cm. wide along the partitions constituted about 16 per cent of the total area available for the nests. If the twenty-six nests built by females separated from males by opaque partitions were distributed at random, from zero to 38 per cent of the nests might be expected to fall in the 5-cm. strip adjacent to the partition.[4] Hence, the 23.1 per cent of the nests located near the partition in one experimental situation of visual isolation and the 12.0 per cent in the second (Table 4) cannot be considered as due to any special attraction to this part of the aquarium.

Nests built by the males are also recorded in Table 4. When the male could see the female, nests could be identified in from 23.8 to 36.3 per cent of the spawnings. All these nests were located near the transparent partition or wall. The somewhat higher percentage of nests built by males separated from females by the transparent grating cannot be considered significant ($P = 0.05$) because of the variability and smallness of the sample. However, it still may be

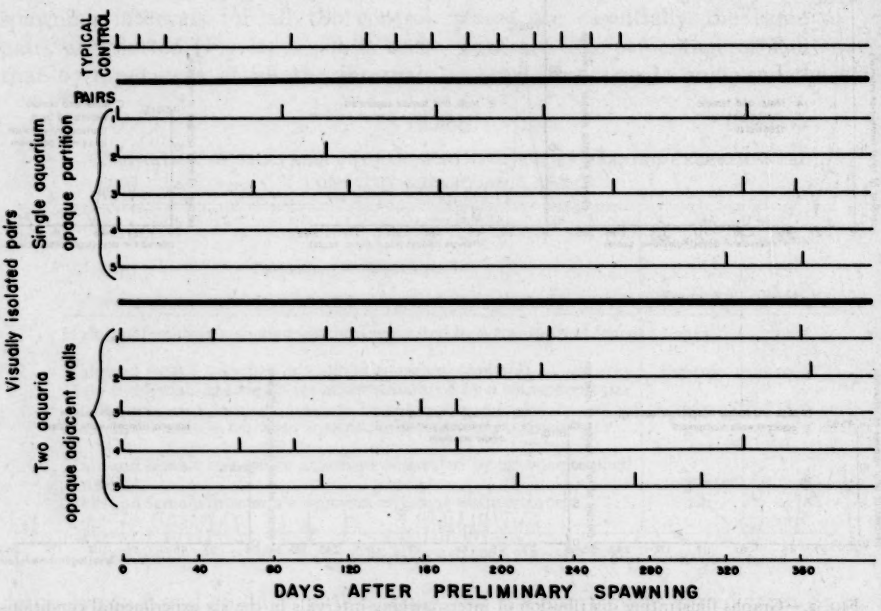

FIG. 3.—Record of successive spawnings of the visually isolated pairs in room B compared with a typical control pair.

[4] Based on sampling limits.

more than coincidental that females in the same experimental situation had the highest spawning frequency.

Only one nest was accounted for by the males visually isolated from the females. This nest was located in the center of a completely isolated aquarium, and its construction was such that there was some doubt as to its proper identification.

TABLE 4

LOCATION OF NESTS BUILT BY MALES AND BY FEMALES

Experimental Conditions	Total No. of Spawnings	Nests Built by Females				Nests Built by Males			
		Spawnings in Which Nests Were Seen		Nests near Partition or Adjacent Wall*		Spawnings in Which Nests Were Seen		Nests near Partition or Adjacent Wall*	
		No.	Percentage of Total No. of Spawnings	No.	Percentage of Total No. of Nests Seen	No.	Percentage of Total No. of Spawnings	No.	Percentage of Total No. of Nests Seen
Male and female isolated but within sight of each other:									
In one aquarium, separated by transparent fence	102	91	89.2	90	98.9	37	36.3	37	100.0
In one aquarium, separated by transparent partition	69	65	94.2	64	98.5	14	20.3	14	100.0
In two adjacent aquaria	59	51	86.4	48	94.1	14	23.8	14	100.0
Male and female visually isolated:									
In one aquarium with opaque partition	26	26	100.0	6	23.1	0	0	.....	.....
In two aquaria with adjacent walls opaque	28	25	89.3	3	12.0	1	3.6	0	0

* Edge of nest within 5 cm. of partition or adjacent wall.

DISCUSSION

In this experiment we have measured the frequency of oviposition by the appearance of eggs, either in the nest or in the mouth of the male or female. The inference is made that these egg-layings are an index to prior ovulation. The mechanism of ovulation has been studied in only one teleost, namely the cyprinodont, *Oryzias latipes* (Robinson and Rugh, 1943). These authors observed that the process is similar to that described for the frog (Rugh, 1935), except that in the fish the eggs are released into the lumen of the ovary rather than into the body cavity. Mature eggs are not stored in the ovary for more than a few hours. Normally they are laid almost immediately after ovulation.

A preliminary investigation by the author of the time of ovulation in *Tilapia* indicates that oviposition occurs within a few hours after ovulation. Repeated failures to obtain *Tilapia* eggs by stripping indicates that females do not carry ovulated or oviductal eggs for any great length of time.

Hartman (1939) has suggested that "all vertebrate classes can probably be

divided like mammals into two groups, those that do, and those that do not require sex stimulation for ovulation and oviposition." *Tilapia* appears to represent a third or intermediate category, in which ovulation occurs spontaneously in complete isolation but where the presence of a male stimulates the ovary (probably indirectly via the optic centers and anterior pituitary) to the extent that ovulation and oviposition occur more frequently. The elaborate courtship patterns characteristic of a number of teleost families suggest that many species belong to this intermediate class. However, this awaits further investigation.

The bulk of the data supporting Hartman's concept comes from observations on birds and mammals. But there are several lines of evidence indicating that even here the line between spontaneous and nonspontaneous ovulators may have been drawn a little too sharply.

The rat is often cited as an example of a spontaneously ovulating mammal. However, the length and character of the oestrous cycle may be affected by copulation (Young, 1941). In animals which were allowed to mate at the beginning of heat, the average length of heat was slightly but significantly shorter (Blandau, Boling, and Young, 1941). Again, Farris (1942) found that in Wistar albino rats ovulation occurs earlier in the oestrous period when preceded by mating. Ball (1934), using sterile female rats, demonstrated a quantitative relationship between the amount of stimulation by the male (number of copulations and ejaculations) and the initiation of pseudo-pregnancy, and Young (1941) suggests that a similar relationship may exist between the magnitude of stimulation by the male and the duration of oestrus.

Similar shortening of the oestrous period following mating has been reported for the guinea pig (Stockard and Papanicolaou, 1919; Avery, 1925; Young, Dempsey, and Meyers, 1935). Stockard and Papanicolaou (1919) state that copulation tends to hasten ovulation; but Young, Dempsey, and Meyers (1935) obtained data to the contrary. The latter authors feel that this subject has not been investigated satisfactorily.

Sterile copulation in sheep tends to shorten the duration of oestrus (McKenzie and Terrill, 1937); and while ovulation occurs spontaneously at each of the heat periods during the early part of the sexual season, coitus is necessary to cause ovulation toward the end of the season (Marshall, 1903). Moreover, according to this investigator, when coition occurs at the beginning of an oestrous period, ovulation takes place a few hours earlier than it otherwise would.

In the cow, ovulation may be hastened and oestrus shortened by service (Weber, 1910–11; Hammond, 1927; Quinlan, Bisschop, and Adelaar, 1941). "Heckling and teasing" by the male mink is believed to induce early oestrus in this species, according to Enders (personal communication to Dr. Frank A. Beach). In subsequent studies of the mink this investigator observed that the largest ovarian follicles of isolated females were smaller and less numerous than those of unisolated individuals who could see other female mink through a wire fence.

Since the presence and activity of the male may, in certain instances, hasten the onset and termination of oestrus, induce and alter the time of ovulation, and initiate the conditions of pseudo-pregnancy and pregnancy, it follows that the total number of ovulations (frequency of ovulation) per breeding season, or per annum of some of the so-called "spontaneously ovulating" mam-

mals, may be considerably altered thereby,

When considering those mammals which ovulate after coitus or comparable sexual stimulation, it is noted that occasional spontaneous ovulations have been recorded for the rabbit, ferret (see table by Long and Evans, 1922, pp. 7–10), and cat (Sadler, quoted by Evans and Swezy, 1931).

Except for the gallinaceous birds it is rare for ovulation to take place spontaneously in this vertebrate class (Marshall, 1942). The domestic hen can apparently lay in complete isolation; and, as Hartman (1939) points out, the same might be true of other domestic fowl, but controlled experiments are lacking. Among the nonspontaneous ovulators, isolated owls, parrots, and some other birds are known to oviposit occasionally (Marshall, 1942), thus paralleling the condition found in mammals.

Amplexus in Anura is often regarded as stimulating ovulation. This concept is based upon an apparently unconfirmed statement by Nussbaum (1895–97). In at least two species, *Rana pipiens* and *Xenopus laevis*, ovulation must precede amplexus (Noble and Aronson, 1942; Aronson, 1944). This does not exclude the possibility of sexual exteroceptive factors influencing ovulation. The frog and toad chorus may very well act in this capacity, since female frogs and toads brought into captivity prior to the breeding season generally fail to ovulate. Moreover, amplexus is a requisite for normal oviposition in *R. pipiens* and *X. laevis* (Noble and Aronson, 1942).

While it is convenient to talk of spontaneously and nonspontaneously ovulating vertebrates, in formulating a working hypothesis it is better to consider the relative influence of sexual exteroceptive stimulation as one member of a group of external and internal factors which normally combine to govern ovarian activity. Although information on this subject is rather scant, it is likely that vertebrate species could be arranged in a series, starting with those animals in which the ovarian mechanism is not influenced or only slightly influenced by stimulation derived from members of the same or opposite sex and placing at the opposite end of the series those species in which ovulation is wholly dependent upon stimulation by the male or a second female. Between these two extremes one could predict innumerable gradations of sexual exteroceptive influences.

The conclusion reached here that the sexual exteroceptive stimuli influencing ovulation are predominately visual, with possibly a slight chemical or vibratory influence, is in agreement with the findings of other investigators of cichlid behavior. Noble and Curtis (1939) observed that in the jewel fish, *Hemichromis bimaculatus*, mating responses are synchronized predominately by visual clues. While it is recognized that other sensory factors may reinforce the visual stimulation, these authors conclude that "touch or other sensory modalities cannot function during courtship vicariously for vision in the way olfaction may function during food seeking."

Seitz (1940) studied the "releaser" mechanism of a related cichlid mouthbreeder, *Astatotilapia strigigena*. Although the breeding behavior of this mouthbreeder is considerably different from that of *Tilapia*, the "releaser" stimuli which are credited with evoking subsequent phases of the fighting and mating patterns in *Astatotilapia* were found to be primarily visual, with chemoreception playing a minor role.

SUMMARY AND CONCLUSIONS

To examine the influence of stimuli provided by the male on the spawning frequency of the female, pairs of *Tilapia* were placed in experimental situations which eliminated visual, vibratory, chemical, and contact stimulation in varying combinations. The experiment revealed that many completely isolated females will continue to spawn at infrequent intervals and that the sight of a male markedly increases this spawning frequency. There is also a possibility that chemical or vibratory stimuli increase slightly the oviposition rate. Since egg-laying is an indicator of prior ovulation, it is seen that *Tilapia* is intermediate between spontaneous and nonspontaneous ovulators. It is suggested that, instead of dividing vertebrates into those that do and those that do not ovulate spontaneously, the relative influence of sexual exteroceptive stimulation should be determined along with the other external and internal factors controlling ovarian activity.

Under conditions in which the male was visible to the female, a semblance of ovarian rhythm was demonstrable. This consisted of a series of short cycles of about 15 days' duration, followed by one or more long cycles. These long cycles sometimes lasted many months. Visual isolation abolished most of the short cycles.

When males and females were separated by transparent partitions, nest construction was often undertaken by both sexes. These nests were almost always located near the partition. When the pairs were separated by opaque partitions, only females built nests, and the nest sites appeared to be chosen at random. This strengthens the conclusion that in *Tilapia* the sexual exteroceptive stimuli influencing the frequency of ovulation are predominately visual.

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ANTIGENIC RELATIONSHIPS IN THE GENUS TETRAHYMENA[1]

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THE relationships of the "*Glaucoma-Colpidium*" group of ciliates have been discussed considerably in the literature of the past few years, even after Furgason (1940) pointed out the essential morphological similarities of the forms and placed them all in the single genus *Tetrahymena*. Recently Kidder and Dewey (1945) examined six strains of *T. geleii* and two strains of *T. vorax* and reported their biochemical differences and similarities. Biochemical reactions in this instance refer to the ability or the lack of ability of the ciliates to ferment certain carbohydrates, their ability or lack of ability to synthesize certain amino acids, growth factors, etc., and their specific responses to chemical conditions in general. It is recognized that organisms may change in their biochemical reactions; and these changes are usually found to be a loss of an enzyme, although less frequently gain variations are found. Variation in Protozoa is to be expected, and Kidder and Dewey (1945) pointed out that biochemical variation had taken place between a parent strain and a daughter strain of *T. geleii* (strains T and T-P) during a few years of separation.

Serological methods are usually regarded as more exact than any other means of tracing relationships between organisms, but these methods have not

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been used extensively with protozoans. Earlier attempts to trace antigenic relationships between protozoans have been open to criticism because the ciliate cultures used were not free from bacteria. Members of the genus *Tetrahymena*, from bacteria-free cultures, were investigated serologically by Robertson (1939*a*, *b*) and by Tanzer (1941). Robertson studied the reaction of five strains of *Tetrahymena* (*"Glaucoma-Colpidium"*) in normal serum of laboratory animals and in serums of rabbits immunized with the ciliates. In relatively low dilutions of the normal serums and in high dilutions of the immune serums the ciliates were markedly affected. In immune serums the ciliates were immobilized, shed a sheath of transparent material from the subcuticular layer with subsequent agglutination, or were killed with lysis. Robertson found that heated normal serum did not react on the ciliates but that heated immune serum reacted in dilutions as high as "1:3,200–1:6,400" in the absence of complement. In the presence of guinea-pig complement the same immune serum had a titer of "1:25,000–1:100,000". Her serum dilutions were made with 0.5 per cent saline, and her test organisms were in 0.2 per cent peptone in 0.5 per cent saline.

Tanzer (1941) studied the action of normal and immune serum on three strains of *Tetrahymena geleii* (see Kidder and Dewey, 1945, for a discussion of the specific strains used). In normal and inactivated normal serum Tanzer's results are in agreement with those of Robertson. In inactivated immune serum, however, Tanzer obtained titers of only 640, 160, and 40.

Both Robertson and Tanzer found rather close relationships between the strains of ciliates from immobilization tests in homologous and heterologous antiserums. Cross-adsorptions were not carried out with the group of ciliates in question.

Work on the antigenic relationships of three strains of *T. geleii* (strain H, E, and T-P) was begun in this laboratory in the summer of 1938. Rabbits were immunized, and the results obtained with normal and immune serums were similar to those reported by Robertson. In inactivated immune serums titers of from 1:5,120 to 1:10,240 were obtained, while the use of guinea-pig complement increased these titers to from 1:40,960 to 1:81,920. Immobilization tests of the different strains in the different immune serums showed a close antigenic relationship between the strains. Moreover, these relationships were confirmed by limited adsorption tests. This study was discontinued, owing to the pressure of other work; but, fortunately, antiserums against *T. geleii* H and *T. geleii* T-P were preserved and were available for the present resumption and extension of the work. Because Kidder and Dewey (1945) found that the six strains of *T. geleii* could be differentiated from one another and that the two strains of *T. vorax*, while differing slightly from one another, were distinct from *T. geleii*, it was decided to resume the investigation of the antigenic relationships of these ciliates.

MATERIAL AND METHODS

Organisms.—Six strains of *T. geleii* and two strains of *T. vorax* were used in this study. These strains have been designated *T. geleii* H, E, T, T-P, W, and GHH and *T. vorax* V and PP; and the source of each is to be found in an earlier report (Kidder and Dewey, 1945). Stock cultures were maintained in 2.0 per cent proteose-peptone broth in distilled water, but for serological work it was

necessary to acclimatize the ciliates to saline. This was accomplished by single consecutive transplants from the stock medium into 2.0 per cent proteose-peptone broth containing amounts of NaCl increasing from 0.2 to 0.8 per cent (0.2, 0.4, 0.6, 0.8). After acclimatization stock cultures for antigens were maintained in 0.8 per cent saline medium.

Preparation of antiserums.—Fifteen milliliters of heavy 4-day-old cultures were concentrated by centrifugation, and the ciliates washed three times in 0.85 per cent saline. The ciliates were suspended in 2 ml. of saline and injected into the marginal vein of the ear of the rabbit. Increased doses were given at 5-day intervals for from three to four injections. Preliminary tests with the immune serums prepared in 1938 showed that marked changes in the antibodies had occurred with age. It was evident from this that two objectives had to be considered in this work: first, the antigenic relationships of the ciliates and, second, the nature of the antibodies (to be discussed in a subsequent report). When strictly fresh antiserums were required for use, the animals were reinjected with their respective antigens. Two to three days after reinjection the animals were bled for 10–20 ml. of serum. If the titer of this serum was approximately the same as the original bleeding after the regular course of immunization, the residue after a particular experiment was preserved and pooled with serum from the first bleeding. Accordingly, the pooled serum was being constantly increased with immune serum of approximately the same titer. These pooled antiserums were used exclusively for the study of antigenic relationships. Preservation was with phenol-ether (equal portions of phenol and ether by weight; 0.009 ml. phenol-ether per milliliter of serum) except for samples of each antiserum from the first bleeding, which were sterilized by Seitz filtration. All serums were stored at 2° C.

Preparation of test antigens.—In the early work considerable difficulty was experienced in obtaining satisfactory antigens for the tests. Ciliates not acclimatized to saline were immobilized in the controls, while tests set up with distilled water gave very low titers. The age of the antigen was of importance, since ciliates in the logarithmic phase of growth are readily injured merely by washing.

After many experiments it was found that ciliates acclimatized to 0.8 per cent saline medium, which had grown for 96 hours at 25 °C., gave uniform results. Under these conditions the ciliates were well into their stationary phase of growth (Kidder, 1941). The ciliates were grown in 125-ml. Erlenmeyer flasks in 50 ml. of medium. Fifteen milliliters of these cultures were concentrated by centrifugation and the supernatant fluid removed. The ciliates were then suspended in 45 ml. of 0.6 per cent saline in 125-ml. Erlenmeyer flasks. These ciliates constituted the test antigens.

Adsorption.—Ciliates used for adsorption were grown for 4 days at 25 °C. in the 0.8 per cent saline medium (to which had been added 0.025 per cent Difco yeast extract) in 1-liter volumes in 3-liter Florence flasks. For each adsorption 60 ml. of the heavy cultures were concentrated by centrifugation, and 3 ml. of a 1:20 dilution of the immune serum were added to the packed ciliates. After suspending the ciliates in the serum the adsorption was allowed to continue for 20 minutes at room temperature. The adsorbent was packed and the serum

drawn off; the adsorption was repeated three to six times.

Tetrahymena is a powerful swimmer, and difficulty was encountered in obtaining cell-free adsorbed serum even when the serum was drawn off as rapidly as possible after centrifugation. This was particularly true after the second or third adsorption with homologous antigens and antiserums and after all adsorptions with some of the heterologous antigens. This difficulty was overcome by placing the tubes in cracked ice for 2 minutes just prior to centrifugation. This treatment completely immobilized the ciliates, and the serum could be drawn off close to the packed mass.

Technique of tests.—Suitable serial dilutions of the normal and immune serums were made in 0.5-ml. quantities in 10 × 100-mm. serological tubes. When two or more antigens were to be tested in one antiserum, master-dilutions were used to prepare the individual tests. The test antigen was added in 0.1-ml. quantities to each tube and to the control tube (0.5 ml. of saline). When complement was employed in the test, the same amount of antigen was added to a control tube containing 0.5 ml. of saline and 0.1 ml. of the complement dilution. Where direct comparisons were to be made of the action of an antiserum with and without complement, 0.1 ml. of saline was added to each tube in the test without complement to equalize the total volume of 0.7 ml. in each test. The complement was always added before the antigen, and the tubes were shaken after the complement was added and again following the addition of the antigen. Tests were incubated at room temperature and were read after varying time intervals, to be noted later, with aid of a dissecting binocular microscope at 15 × magnification.

Action of serum on ciliates.—When ciliates come into contact with immune serum, they undergo changes, which, if the serum is of sufficient strength, result in their death. The first evidence of reaction is a gradual slowing of motion, then the complete immobilization of increasing numbers of ciliates, and finally death. Death may be accompanied by agglutination, owing to the exuding of gelatinous materials (the sheath of Robertson, 1939*a*), or in some instances by lysis. We have made no attempt in this study to separate the specific types of responses to various conditions, as was done by Robertson (1939*a*), but have used immobilization and death as evidence of the reactivity of our serums. Tanzer (1941) also used immobilization as the end point in his investigation of antigenic relationships in *Tetrahymena*. Following the procedure of most serological reports, we have used the numbers 0–4 to denote the intensity of the response. The figure 4 represents complete killing; 3 indicates that only a few ciliates (perhaps 1 per cent) were not killed; 2 means that approximately 50 per cent were killed; 1 indicates no killing but partial immobilization (slowing of motion); 0 represents no reaction. With practice it is possible to read the tests with considerable speed and accuracy.

RESULTS

The limited work in 1938 and the more extensive present investigation confirm and extend the findings of Robertson (1939*a*). The normal serums of animals, including man, are capable of immobilizing, agglutinating, killing, and occasionally causing the lysis of *Tetrahymena*. The active substance in such serums was found to be relatively weak, however, as a positive reaction seldom occurred beyond a dilution of 1:160. When in-

activated (heated at 55° C. for 30 minutes), these serums were entirely devoid of activity on the ciliates.

Seven of the eight strains of *Tetrahymena* were tested in the normal serums of seven rabbits selected for immunization (*T. geleii* T-P was not used). The tests (in dilutions of 1:10 to 1:320) were set up as previously described and were read after 1 hour and again after 2 hours. The potency of the serums varied to some extent. On the other hand, the susceptibility of the different strains also varied, judging by the response of the ciliates to the action of a given serum. In one serum, for example, *T. geleii* E and H were negative after 2 hours; strain T showed a reaction in a dilution of 1:10 and strain W in the 1:20 dilution; strain GHH was affected in the 1:160 dilution; and, while *T. vorax* V and PP reacted only in the 1:10 dilution, there was considerable lysis of these ciliates. This last represents one of the few occasions where lysis occurred in normal serum. The average titer of the different serums was 1:10.

Titers of the immune serums varied markedly with the time between injection and bleeding, to some extent with the presence or absence of complement in the test and with the age of the serum. With fresh antiserums and in the presence of guinea-pig complement, titers usually ranged from 1:20,480 to 1:163,840. On one occassion with a fresh *T. geleii* W antiserum the homologous titer was 1:655,360. In the absence of complement the titers of the fresh antiserums ranged from 1:5,120 to 1:40,960. However, the antiserums prepared in 1938 against *T. geleii* H and *T. geleii* E gave only a weak reaction (partial immobilization; see caption of Table 2) in dilutions of from 1:40 to 1:80 without complement and gave titers of 1:40,960 with complement. Fresh inactivated immune serum without complement usually gave titers only one, two, or three dilutions lower than the titers obtained with the same serum in the presence of complement.

It is apparent from the foregoing that the antibodies or the complement fraction utilized by the antibodies differs from the commonly accepted amboceptor-complement reaction. Considerable evidence has been collected to show that the complement fractions which cause hemolysis of the sensitized sheep cell are not the same as those which function in the ciliate antigen-antibody reaction. A subsequent report will be made on the nature of the reaction between ciliate antigen-antibody and complement.

Antigenic relationships.—The eight strains of *Tetrahymena* were tested individually in the eight antiserums. A summary of the results is shown in Table 1. It is apparent that the two species of *Tetrahymena* divide themselves into two antigenic groups. It is obvious that there is a marked continuity of antigens within each of the two groups. It is quite impossible to determine homologous antigen and antiserum from Table 1. Antigen E, for example, reacts in the five heterologous antiserums of its group (*T. geleii*) much as the five heterologous antigens react in antiserum E. A similar condition holds for the antigens and antiserums of *T. vorax*.

In checking the weak reactions of antigen V in antiserums against strains W and GHH, antigen PP, and antiserum E (Table 1) it was found that, if readings were made over longer time intervals (1½–3 hours), the strains of *T. geleii* in *T. vorax* antiserums and the strains of *T. vorax* in *T. geleii* antiserums did react. Marked difficulties were encountered in getting exact titers and in duplicating

results in the cross-immobilization tests, since the ciliates frequently were affected in the complement controls over the long period. It can be definitely stated, however, that strains of the *T. geleii* group and the *T. vorax* group possess common, against one of the antigens is presumptive evidence of antigenic identity. True antigenic identity, however, can be established only by adsorption tests. Accordingly, each of the eight antiserums was adsorbed separately with each of

TABLE 1

SUMMARY OF THE REACTIONS OF EIGHT STRAINS OF *Tetrahymena* IN EIGHT ANTISERUMS

Each serum represents final pooling from several bleedings. Guinea-pig complement was added in a final dilution of 1:80. The first figure in each case represents the final titer, while the second figure represents the last dilution where complete killing occurred. Tests read after 30 minutes.

Antigen	Antiserum							
	E	H	T-P	T	W	GHH	V	PP
H	40,960 2,560	40,960 10,240	40,960 2,560	40,960 2,560	81,920 10,240	163,840 10,240	No reaction	No reaction
E	40,960 2,560	40,960 2,560	40,960 2,560	40,960 2,560	40,960 10,240	40,960 10,240	No reaction	No reaction
T-P	40,960 2,560	40,960 2,560	40,960 2,560	163,840 2,560	40,960 640	40,960 10,240	No reaction	No reaction
T	20,480 2,560	10,240 2,560	81,920 2,560	40,960 2,560	20,480 2,560	40,960 2,560	No reaction	No reaction
W	40,960 2,560	40,960 2,560	40,960 640	40,960 2,560	20,480 2,560	163,840 10,240	No reaction	No reaction
GHH	40,960 2,560	40,960 2,560	40,960 640	40,960 640	40,960 2,560	163,840 10,240	No reaction	No reaction
V	No reaction	No reaction	No reaction	No reaction	40 No killing	40 No killing	81,920 10,240	40,960 2,560
PP	40 No killing	No reaction	No reaction	No reaction	No reaction	No reaction	40,960 2,560	40,960 2,560

although probably minor, antigens. It is significant that, while titers as high as 1:2,560 were found, complete killing was not encountered even in the 1:10 dilutions. Exact antigenic relationships between the strains of *T. geleii* and the the strains of *T. vorax* must be left for further study.

Adsorptions.—The reaction of two antigenic substances to the same titer (homologous titer) in a serum prepared the eight antigens. In the first set of adsorptions the antiserums were treated with the standard amount of antigen (packed cells from 60 ml. of 4-day-old cultures) for each of the adsorptions. In later adsorptions twice the standard amount of antigen was used for the first adsorption; thereafter the standard amount. After adsorbing an antiserum with any antigen the adsorbed antiserum was tested with the organism

homologous to the antiserum and occasionally with other antigens.

To obtain all possible information, all antiserums were tested after the first, second, third, and fifth adsorptions. After a number of such tests the general antigenic pattern of the organisms became apparent, and testing after adsorptions was reduced to the third and fifth.

Adsorption tests confirmed the immobilization tests in grouping the eight strains of organisms into two types (*T. geleii* and *T. vorax*). Of the six strains of *T. geleii*, however, no two were antigenically identical in every respect. Strains E, H, T-P, W, and GHH appear to be qualitatively identical but differ quantitatively. That is, these five strains all possess the same antigens; but the amount or concentration or distribution of the antigenic materials varies in the individual strains. Table 2 illustrates this point. In antiserum prepared against strain H the homologous antigen gave a titer of 1:40,960. After one adsorption with *T. geleii* H antigen the titer dropped to 1:160; after a second adsorption, to 0. On the other hand, after one adsorption with *T. geleii* E antigen the homologous titer of the same antiserum was 1:2,560; after the third adsorption, 1:640; it was negative only after the fifth. It will be noted that three adsorptions with either *T. vorax* V or PP failed

TABLE 2

ANTISERUM PREPARED AGAINST *T. geleii* H, ADSORBED WITH EIGHT ANTIGENS (*T. geleii* H, E, T-P, T, W, AND GHH AND *T. vorax* V AND PP)

Tested with *T. geleii* H. Zero = no reaction; 1 = partial immobilization; 2 = approximately 50 per cent completely immobilized or dead, all others partially immobilized; 3 = most dead or completely immobilized, a few (approx. 1 per cent) feebly moving; 4 = all dead or completely immobilized. One-hour readings.

Adsorbing Antigen	No. of Adsorptions	Dilution												
		20	40	80	160	320	640	1,280	2,569	5,120	10,240	20,460	40,960	Control
Unadsorbed		4	4	4	4	4	4	4	4	4	3	2	1	0
H	1	1	1	1	1	0	.....	.....	.....	.....	.....	.....	.....	0
	2	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0
E	1	4	4	4	4	4	4	4	2	0	.....	.....	.....	0
	3	1	1	1	1	1	1	0	.....	.....	.....	.....	.....	0
	5	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0
T-P	1	2	2	1	1	1	0	.....	.....	.....	.....	.....	.....	0
	3	1	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0
	5	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0
T	3	1	1	1	1	1	1	1	0	.....	.....	.....	.....	0
	5	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0
W	3	2	2	2	2	1	0	.....	.....	.....	.....	.....	.....	0
	5	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0
GHH	3	1	1	1	1	1	1	0	.....	.....	.....	.....	.....	0
	5	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0
V	3	4	4	4	4	4	4	4	4	4	2	1	0	0
PP	3	4	4	4	4	4	4	4	4	4	2	2	0	0

to reduce significantly the homologous titer of the antiserum (a one- or two-tube reduction of titer can be accounted for by dilution of the antiserum during adsorption, owing to the fact that the adsorbing ciliates cannot be packed solidly).

Tetrahymena geleii T differs from the other five strains of the species since it possesses a minor antigen not found in the other five. Table 3 shows that, while one adsorption of antiserum T with antigen T removed all homologous immobilizing substance, five adsorptions with antigens E, H, T-P, W, and GHH, though markedly reducing the homologous titer, failed to remove all of the immobilizing substances for antigen T. It will again be noted that five adsorptions with either *T. vorax* V or PP failed to reduce the homologous titer of antiserum T by more than one dilution.

As with antiserums H and T, five adsorptions of antiserums E, T-P, W, and GHH with either *T. vorax* V or PP failed to reduce the homologous titer more than one dilution.

Table 4 shows that *T. vorax* V and PP appear to be antigenically identical, both qualitatively and quantitatively, since the homologous antibodies were removed from either antiserum almost equally by the heterologous strain.

TABLE 3

ANTISERUM PREPARED AGAINST *T. geleii* T, ADSORBED WITH EIGHT ANTIGENS (AS IN TABLE 2)

Tested with *T. geleii* T. One-hour readings

Adsorbing Antigen	No. of Adsorptions	Dilution												
		20	40	80	160	320	640	1,280	2,560	5,120	10,240	20,460	40,960	Control
Unadsorbed		4	4	4	4	4	4	4	4	4	3	2	1	0
T	3	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0
E	3	4	4	4	3	2	2	0	.....	.....	.....	.....	.....	0
	5	4	4	4	3	2	1	0	.....	.....	.....	.....	.....	0
H	3	4	4	3	2	2	1	0	.....	.....	.....	.....	.....	0
	5	4	4	3	2	1	0	.....	.....	.....	.....	.....	.....	0
T-P	3	4	3	3	2	2	1	0	.....	.....	.....	.....	.....	0
	5	3	2	2	1	1	0	.....	.....	.....	.....	.....	.....	0
W	3	4	4	3	2	2	1	1	0	.....	.....	.....	...	0
	5	4	4	3	2	1	0	.....	.....	.....	.....	.....	.....	0
GHH	3	4	4	3	2	1	1	1	1	0	.....	.....	.....	0
	5	4	4	3	2	1	0	.....	.....	.....	.....	.....	.....	0
V	5	4	4	4	4	4	4	4	4	2	1	0	.....	0
PP	5	4	4	4	4	4	4	4	4	2	1	0	.....	0

Because of the slow reaction of some strains of *T. geleii* in *T. vorax* antiserums (Table 1), antiserums V and PP were adsorbed three times for three-hour intervals with double concentrations (organisms from 120 ml. of 4-day cultures) of antigens E, H, T, and GHH. Homologous titers of the adsorbed antiserums were not reduced significantly.

DISCUSSION

Antigenic similarities may or may not be correlated with other biochemical simi-

larities. Thus, among the bacteria, many strains may show the same reactions in the standard biochemical tests and yet be antigenically distinct. Conversely, antigenically identical strains may differ in biochemical reactions. It is of interest, therefore, to note that all six strains of *T. geleii* studied differ from one another *T. geleii*. It was found possible to separate the two species of *Tetrahymena* on the basis of either of these biochemical reactions (Kidder and Dewey, 1945), and the serological results confirm our original designations.

Tetrahymena vorax PP differs from V only in minor biochemical reactions,

TABLE 4

ANTISERUM PREPARED AGAINST *T. vorax* V, ADSORBED WITH *T. vorax* PP

Tested with *T. vorax* V. One-hour readings

No. of Adsorptions	Dilution														
	20	40	80	160	320	640	1,280	2,560	5,120	10,240	20,480	40,960	81,920	163,840	Control
Unadsorbed	4	4	4	4	4	4	4	4	4	4	3	2	2	1	0
Adsorbed 1	4	4	4	4	4	3	3	2	2	2	0	.....	.....	.....	0
Adsorbed 2	1	1	1	1	1	1	0	.....	.....	.....	.....	.....	.....	.....	0
Adsorbed 3	1	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0
Adsorbed 5	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0

ANTISERUM PREPARED AGAINST *T. vorax* PP, ADSORBED WITH *T. vorax* V

Tested with *T. vorax* PP. One-hour readings

No. of Adsorptions	Dilution														
	20	40	80	160	320	640	1,280	2,560	5,120	10,240	20,480	40,960	81,920	163,840	Control
Unadsorbed	4	4	4	4	4	4	4	4	4	4	3	2	1	0	0
Adsorbed 1	4	4	4	4	4	3	2	2	2	1	0	.....	.....	.....	0
Adsorbed 2	1	1	1	1	1	0	.....	.....	.....	.....	.....	.....	.....	.....	0
Adsorbed 3	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0

both biochemically (Kidder and Dewey, 1945) and antigenically, while the two strains of *T. vorax* show slight biochemical differences but are antigenically identical. The biochemical differences within each species are less marked than between the species, and the antigenic relationships follow the same pattern. *Tetrahymena vorax* V and PP both possess lactase, while lactose is not fermented by any of the strains of *T. geleii*. The amino acid glycine is stimulatory to *T. vorax* and either neutral or inhibitory to principally the ability of the former to synthesize valine under certain conditions. This difference may depend, however, on the balancing of the concentrations of other amino acids in the medium and may reflect a difference of level of sensitivity between the strains. Within the species *T. vorax*, as judged by the two strains studied, there is more biochemical homogeneity than within the species *T. geleii*. This holds true for their antigenic homogeneity.

From a biochemical standpoint, *T.*

geleii E appears to differ most widely from the other five strains. It fails to grow normally in the "nineteen amino acid" medium. This failure is due to susceptibility to inhibitory amino acids, for it gives optimal growth with a smaller number (the ten indispensable amino acids plus serine). It resembles the strains of *T. vorax* in its ability to ferment galactose. These differences are reflected in the antigenic makeup of this strain only in a quantitative way.

Perhaps the most interesting relationship brought out by the biochemical study (Kidder and Dewey, 1945), and substantiated by the serological investigation, is that existing between *T. geleii* T and T-P. Strain T is the parent strain, isolated by Thomas in 1931. Strain T-P is the daughter strain of T separated from it in 1934. During the period of separation there have occurred biochemical variations which have become fixed. Thus, strain T-P fails to grow normally in the "nineteen amino acid" medium but gives optimal growth in the "eleven" (the ten indispensable amino acids plus serine). Strain T grows optimally in both types of medium. Moreover, strain T-P is stimulated by cystine, while strain T is not. These, and other minor differences noted, represent true variations; but it is impossible to decide which strain varied, since no biochemical information is at hand regarding the parent strain before the separation. From the serological studies it becomes apparent that the variations of a biochemical nature are reflected in, or coincident with, changes in the antigenic structure, for strain T is found to have all the antigens of strain T-P plus additional antigens. Still it is impossible to designate the variant strain. It might be argued that, inasmuch as loss variation is much more common than gain variation, strain T-P has varied from the parent strain by losing some of its antigenic structure. On the other hand, the fact that strain T differs from all other five strains of *T. geleii* in the possession of qualitatively different antigens might represent a variation from the "normal" *T. geleii* makeup. In spite of the fact that gain variations are the exception to the rule, we feel that the chances that T-P has varied by losing antigens which make it, therefore, qualitatively identical with strains E, H, W, and GHH are less than the chances that strain T has varied by gaining new antigens. This becomes more plausible when it is noted that the origins of the various strains represent widely scattered localities. Strain H and T were both isolated at Pacific Grove, California; strain E at New York City; strain W at Woods Hole, Massachusetts; strain GHH at Memphis, Tennessee. It is important to note, in this connection, that *T. vorax* V was isolated in Cumberland, Rhode Island, while *T. vorax* PP was isolated in Memphis, Tennessee, and yet they are antigenically nearly identical.

SUMMARY

1. Six strains of *T. geleii* and two strains of *T. vorax* have been examined by cross-adsorption techniques for antigenic relationships.

2. Five of the strains of *T. geleii* possess qualitatively identical antigens, but all differ quantitatively (amount, concentration, or distribution of antigenic materials). Strain T possesses all the antigens of the other five strains plus additional antigens.

3. The two strains of *T. vorax* appear to be nearly identical antigenically.

4. Marked antigenic differences were

found between the two species, as indicated by the absence of cross-reactions within the standard testing time.

5. The serological studies confirm the earlier biochemical studies in proving that a variation has occurred between *T. geleii* T (parent) and *T. geleii* T-P (daughter), and the evidence seems to support the hypothesis that strain T has varied by gaining antigens.

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MINERAL DEFICIENCY AS A FACTOR LIMITING GROWTH OF THE CILIATE TETRAHYMENA GELEII

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DURING the course of some work on vitamin requirements of *Tetrahymena geleii* a preparation of "vitamin-free" casein was used in one of our basal media. As already reported (Hall and Cosgrove, 1942, 1944), a satisfactory medium could be prepared by adding certain salts to a solution of this casein, whereas the casein alone failed to support growth in serial transfers. Such a casein solution is inadequate also for another strain, *T. geleii* W (Dewey, 1944). These results led us to investigate the mineral deficiency responsible for failure of *T. geleii* to grow in unsupplemented casein medium.

MATERIAL AND METHODS

The strain of *T. geleii* was the one used previously (Hall, 1944; Hall and Cosgrove, 1944) under the name *Glaucoma piriformis*. As pointed out earlier (Hall, 1944), we had retained this older terminology in order to avoid confusion of this strain with others used in our laboratory. Kidder and Dewey (1945), in their comparative study of eight strains, have now proposed satisfactory labels which will prevent confusion. Accordingly, we are designating "*Glaucoma piriformis*" as "*Tetrahymena geleii* GP." In our experimental series the following media

[1] In the absence of W. B. Cosgrove, on active duty overseas, the senior author is responsible for final interpretation of the data presented in this paper.

have been tested, with and without modifications as specified in particular series:

1. Casein Medium

"Vitamin-free" casein (Casein-Harris)	10.0 gm.
Distilled water	1.0 liter

2. Salted Casein Medium

"Vitamin-free" casein (Casein-Harris)	10.0 gm.
$MgSO_4 \cdot 7H_2O$	0.2 gm.
K_2HPO_4	0.2 gm.
$CaCl_2 \cdot 2H_2O$	0.1 gm.
$FeCl_3 \cdot 6H_2O$	0.0025 gm.
Distilled water	1.0 liter

To this solution were added $MnCl_2 \cdot 4H_2O$ and $ZnCl_2$, each in a concentration of 1×10^{-7} gm/cc.

In addition to the salted casein medium, various salts were added singly and in combinations to the plain casein medium. Each such medium was adjusted to the desired pH, tubed in 9.0 cc. amounts in 16 mm. Pyrex culture tubes that were plugged with bleached non-absorbent cotton (Johnson and Johnson), and then sterilized at 122° C. for 20 minutes. The inorganic salts were "analyzed reagent" quality (Mallinckrodt), and all distilled water was redistilled in Pyrex glass before use. In the descriptions of certain series, quantitative references to traces of iron and calcium are based upon calculations from stated analyses of the various salts used.

Casein medium was supplemented also with certain organic preparations. Yeast extract (Difco) was added to a flask of distilled water (1 gm. in 100 cc.), sterilized in the autoclave for 30 minutes, and then added in 1.0 cc. amounts to tubes containing 9.0 cc. of casein medium. The tubes were subsequently sterilized (20 minutes at 122° C.). The same procedure was followed for brewers' yeast (Yeast-Harris) except that this material was added as a suspension to the tubes of casein medium. In the preparation of alfalfa-meal extract, a suspension (5.0 gm. in 100 cc. of distilled water) was sterilized in the autoclave for 30 minutes and then allowed to stand at room temperature for 48 hours. The supernatant liquid was then added in 1.0 cc. amounts to tubes of casein medium before final sterilization. Dethiaminized alfalfa extract was prepared by bringing a similar suspension of alfalfa meal to a boil and then adding NaOH until the pH of the supernatant liquid exceeded 9.6. The material was then heated for 1 hour at 122° C. and allowed to stand for 48 hours at room temperature. Subsequently the pH of the supernatant liquid was lowered with HCl to a satisfactory point and the mixture was again sterilized (20 minutes at 122° C.). The supernatant liquid was then added aseptically in 1.0 cc. amounts to tubes of sterile casein medium.

Cultures were incubated in darkness at 24° ± 0.2° C., except for the last few transfers in Series XIII–XIX, when the incubator remained at room temperature. Counts to determine the density of population (number of ciliates per cubic centimeter) were made as previously described (Hall, Johnson, and Loefer, 1935), with the exception that the ciliates were killed by heat (75°–80° C.) instead of with a fixative in order to avoid precipitation of the casein.

Maximal density of *T. geleii* in our salted casein medium is reached in less than 2 weeks (Hall, 1944); accordingly, tubes were fixed for counting after varying periods within this limit, so as to avoid the normal decrease in population following the phase of maximal density. The counting technique was supplemented by frequent examination of cultures with a stereoscopic binocular microscope. Since the writers were inter-

ested primarily in determining the suitability of different supplemented casein media for growth of *T. geleii*, counts were usually made only as terminal counts in transfers of varying length. Although counts made at intervals during single transfers have shown differences in rates of growth within a given group of series, such data will not be considered here. "Maximal density," as noted for the various experimental series, represents the maximum observed in any transfer after the fourth. The average density for any one series was always somewhat lower, since counts in some transfers were made before the populations reached their maxima.

EXPERIMENTAL RESULTS

The growth of *T. geleii* GP in salted casein medium has been reported previously (Hall and Cosgrove, 1944; Hall, 1944). Casein medium without added salts failed to support growth, whereas the original strain in salted casein medium was maintained for 15 months (28 transfers) and then discontinued. The strain attained an average density of about 35,000. Subsequent findings are in agreement with these earlier observations.

In Series I–IV (Table 1) the use of a dilution flask of sterile casein medium, seeded from a culture in casein-peptone medium, reduced the carried-over peptone to a low concentration in transfer 1. Casein medium (Ser. I, pH 7.3) showed no growth, even in the first transfer. On the other hand, salted casein medium (Ser. II, pH 7.4 in transfers 1–5; pH 6.9 in transfers 6–18) was adequate for growth. Growth was slow in transfers 1–5 but fairly heavy in later transfers. Casein medium with added phosphate (Ser. III, pH 7.1) was no more satisfactory than the basal medium. In salted casein medium with the phosphate omitted (Ser. IV, pH 7.3) growth was a little heavier than in salted casein medium at pH 7.4, but slower than in this medium at pH 6.9 (Ser. II). The results showed that K_2HPO_4 was an inadequate supplement for casein medium and that omission of this salt from salted casein medium did not prevent growth.

Series V–VIII (Table 1) were started from transfer 1 of Series IV. The addition of $FeCl_3 \cdot 6H_2O$ (1×10^{-6} gm/cc) to casein medium (Ser. V, pH 7.1) insured continued growth, although growth was relatively slow. A collateral strain (Ser. V, *A*), started from Series V at transfer 5, failed to survive in unsupplemented casein medium. Growth was maintained after the omission of $FeCl_3$ and K_2HPO_4 from salted casein medium (Ser. VI, pH 7.1) had reduced the added iron to traces (approximately 2×10^{-9} gm/cc) present in the other salts. The omission of K_2HPO_4 and $MgSO_4$ from salted casein medium (Ser. VII, pH 7.1) likewise failed to inhibit growth. On the other hand, after elimination of $CaCl_2$ and K_2HPO_4 from salted casein medium (Ser. VIII, pH 7.1), growth was no better than in Series V with $FeCl_3$ as the only supplement.

At this point our results indicated: (1) that addition of an iron salt converted an inadequate casein medium into one which would support small populations in serial transfers, whereas omission of the iron in a collateral series was followed by death of the strain within three transfers; (2) that omission of the calcium and potassium salts from salted casein medium reduced the population to a density comparable to that with iron as the only supplement; (3) that the omission of the potassium and magnesium salts had no marked effect on growth. Accordingly, it was suspected

that unsupplemented casein medium might be deficient in both iron and calcium. This possibility was considered further.

Series IX–XII (Table 1) were started from transfer 5 of Series IV. Salted casein medium (Ser. IX, pH 6.9) was used for direct comparison with other media in this group. A simplified medium (Ser. X, pH 6.9), containing $CaCl_2 \cdot 2H_2O$ (2×10^{-4} gm/cc) and $FeCl_3 \cdot 6H_2O$ (2.5×10^{-6} gm/cc), was about as satisfactory as salted casein medium. Addition of only the calcium salt (Ser. XI, pH 6.9), which contained a trace of iron, reduced the added iron to approximately 2×10^{-9} gm/cc without decreasing population yield. A collateral series (Ser. XI, *A*), started from Series XI at transfer 7, failed to survive the third transfer in plain casein medium. Omission of the iron and calcium salts from salted casein medium (Ser. XII, pH 6.7), with a consequent reduction of added iron and calcium to traces (3×10^{-9} and 4×10^{-8} gm/cc, respectively), lowered the density of population.

It now appeared that this ciliate requires supplementary calcium, as well as iron, for good growth in casein medium, and that our basal medium (unsupplemented casein) was deficient in iron and contained relatively little available calcium. Substitution of different calcium salts for $CaCl_2$ was next attempted.

Series XIII–XV (Table 1) were started from a stock in salted casein medium (transfer 7). In Series XIII (pH 6.3) $CaCl_2 \cdot 2H_2O$ (1×10^{-4} gm/cc) was added to casein medium; in Series XIV (pH 6.3), $CaSO_4$ (1.1×10^{-4} gm/cc) containing a trace of iron (concentration in the medium, 3×10^{-9} gm/cc); in Series

TABLE 1*

GROWTH IN CASEIN MEDIA

Series	Medium	Final Transfer	Maximal Density	Suitability for Growth
I	CM	O, 3	.....	−
II	SCM	D, 18	40,000	+
III	CM+K_2HPO_4	O, 3	.....	−
IV	SCM−K_2HPO_4	D, 11	20,000	+
V	CM+$FeCl_3$	D, 17	15,000	+
V, *A*	CM	O, 3	.....	−
VI	SCM−($FeCl_3$, K_2HPO_4)	D, 17	32,000	+
VII	SCM−(K_2HPO_4, $MgSO_4$)	D, 17	39,000	+
VIII	SCM−(K_2HPO_4, $CaCl_2$)	D, 13	13,000	+
IX	SCM	D, 13	39,000	+
X	CM+($FeCl_3$, $CaCl_2$)	D, 13	37,000	+
XI	CM+$CaCl_2$	D, 13	45,000	+
XI, *A*	CM	O, 3	.....	−
XII	SCM−($CaCl_2$, $FeCl_3$)	D, 13	12,000	+
XIII	CM+$CaCl_2$	D, 13	15,000	+
XIV	CM+$CaSO_4$	D, 13	11,000	+
XV	CM+[$Ca(NO_3)_2$, $FeCl_3$]	D, 13	15,000	+
XVI	CM+yeast extract	D, 13	46,000	+
XVII	CM+brewers' yeast	D, 13	125,000	+
XVIII	CM+alfalfa extract	D, 13	109,000	+
XIX	CM+dethiaminized alfalfa	D, 8	43,000	+

* CM, case in medium; D, discontinued at indicated transfer; SCM, salted casein medium; +, adequate for growth in serial transfers; −, inadequate for growth; O, viability tests negative at indicated transfer.

XV (pH 6.3), both $Ca(NO_3)_2$ (2×10^{-4} gm/cc) and $FeCl_3 \cdot 6H_2O$ (1×10^{-6} gm/cc). Although the pH of the media and concentration of calcium were lower than in Series XI, growth was maintained in all three series.

These results indicated that $CaSO_4$ (containing a trace of iron) and $Ca(NO_3)_2$ (with an added iron salt) will replace $CaCl_2$ (containing a trace of iron) as a supplement to casein medium under the specified conditions.

Since Kidder and Dewey (1942) had already shown that casein medium, supplemented with dethiaminized extract of alfalfa meal, was satisfactory for *T. geleii* W, attempts were made to substitute organic preparations for inorganic salts. Series XVI–XVIII (Table 1) were started from a dilution flask of casein medium seeded with a stock strain in salted casein medium. Addition of yeast extract (Ser. XVI, pH 6.3), brewers' yeast (Ser. XVII, pH 6.3), and extract of alfalfa meal (Ser. XVIII, pH 6.2) to casein medium produced populations as large as, or significantly greater than, those observed in salted casein medium. Yeast extract was little more effective than the usual salt mixture, whereas brewers' yeast tripled and alfalfa-meal extract more than doubled the population. The maintenance of relatively dense populations for longer periods than in salted casein medium simulated results obtained previously with added vitamins (Hall, 1944; Hall and Cosgrove, 1944). Dethiaminized extract of alfalfa meal (Ser. XIX, pH 6.5), as a supplement to casein medium, produced populations approximating the heaviest observed in salted casein medium, although less than half as dense as those obtained with untreated alfalfa extract (Ser. XVIII). Obviously, the mineral deficiency of our casein medium is eliminated by the addition of dethiaminized alfalfa extract, as well as the untreated organic supplements.

DISCUSSION

Although there is relatively little detailed information concerning particular mineral requirements of Protozoa, calcium and iron seem to be essential for growth of at least several species. For example, *Euglena stellata* (Dusi, 1933) and *Hyalogonium klebsii* (Pringsheim, 1937) have been found to require calcium, while iron is needed for growth of *Polytoma uvella* (Lwoff, 1930), *Astasia* sp. (Schoenborn, 1940), and *Chilomonas paramecium* (Hutchens, 1940). Our results thus add another protozoan to the list of those for which certain mineral requirements have been reported, although the apparent need of *T. geleii* GP for calcium and iron represents nothing unusual in protozoan nutrition. The observation that certain organic preparations used in culture media apparently contain adequate supplies of these minerals while another does not stresses the importance of considering inorganic, as well as organic, foods in investigating nutritional requirements of ciliates.

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NEW BOOKS IN PHYSIOLOGICAL ZOÖLOGY

[This section comprises reviews of selected new books containing new and significant material in the field of physiological zoölogy. Books submitted for review should be sent directly to the review editor: Frank A. Brown, Jr., Department of Zoölogy, Northwestern University, Evanston, Illinois.]

THE PERMEABILITY OF LIVING CELLS,[1] BY S. C. BROOKS AND MATILDA MOLDENHAUER BROOKS

Reviewed by M. H. JACOBS
Department of Physiology, University of Pennsylvania

THE difficulties of scientific publication in time of war are never inconsiderable; but when a manuscript in the course of transformation into a book becomes separated from its authors by a fighting front which finally involves their own country, these difficulties begin to be formidable. When, in addition, further access of the authors to their writings is sternly forbidden by their own government, the situation becomes truly discouraging. The authors of the present volume are to be congratulated on the perseverance they have shown, under the circumstances mentioned, in having finally made it available to their American colleagues before its value has been too greatly diminished by time.

The history of their efforts[2] is an interesting commentary on governmental control of scientific matters. In 1929 the two authors, each of whom had won an independent position of distinction in the field of cell permeability, were invited to contribute a volume to the well-known series of "Protoplasma Monographs." The work was completed in 1939, just before the beginning of the second World War and two years before this country became involved. The first serious difficulties encountered were delays in the correction of the proof incident to the British censorship of mail in Bermuda. Despite these delays, however, the work was finally published in Germany in 1941, and the British Ministry of Economic Warfare gave permission for its passage through England to the country of its origin.

At this point officials of the United States Treasury decided that benefits to the enemy might result if the book were made available to American readers. The obvious procedure in such a case, which was finally adopted after many delays, was to have the book lithoprinted in this country. This could be done, however, only after several years had elapsed and much correspondence, a trip by one of the authors to Washington, and the subsequent intercession of a number of individuals representing various scientific societies and libraries had at last overcome official inertia. In the meantime the material in the book had reached the mature age of five years, which is not far from the normal life-span of many scientific works.

Fortunately, this delay was less serious

[1] Berlin-Zehlendorf: Gebrüder Borntraeger, 1941; Lithoprint by Edwards Bros., Ann Arbor, Mich., 1944. Pp. 396.

[2] *Science*, 100:30, 1944.

than it would have been in a time of greater scientific productivity. While important work in the field of cell permeability has appeared since 1939, the last year represented in the main bibliography of the book, its quantity is relatively small, as compared with that of the preceding six-year period, and little of it has been of a revolutionary nature. Incidentally, the bibliography just mentioned, including as it does some fifteen hundred titles, is not the least valuable feature of the book.

For another reason the present volume is less likely to show its age than some others. It makes no pretense of being a complete, encyclopedic treatment of the subject of cell permeability, but is rather a stimulating discussion by the authors of several topics in this field that have interested them personally, with the intentional omission of others that have not. In a work that is designedly incomplete, the absence of some given new material is much less conspicuous than it would be, for example, in a comprehensive German "*Handbuch.*"

There is something to be said in favor of the present personal, as opposed to the more usual encyclopedic, method of treating a scientific subject, provided only that adequate works of reference—which are not lacking in the field of cell permeability—are available for those who need them. Whether because of agreement or of disagreement with the opinions expressed by the authors, many readers of the present volume will find it more stimulating than a more complete catalogue of facts would have been. By an interesting coincidence, another recent work on permeability, by Davson and Danielli, reviewed in these columns in April, 1945, is of a similar personal nature; fortunately, the two books, as might have been anticipated, supplement, rather than duplicate, each other.

The general interests of the present authors are well shown by the amounts of space allotted by them to various topics. For example, the controversial subject of permeability to strong electrolytes, to which one of the authors has made important contributions, some too recent for inclusion, receives 52 pages, while that of permeability to nonelectrolytes, about which far more is definitely known and which has contributed so much to an understanding of the fundamental nature of the cell surface, is disposed of in 18 pages. In certain respects this greater attention to controversial matters than to well-established facts may be a virtue rather than a fault, though readers of the book who are interested primarily in the latter type of information may at times be disappointed. Such persons would doubtless have welcomed, for example, a fuller discussion of the important work of Collander and his associates, particularly since much of it in the original is not readily accessible to American readers.

A second contrast in treatment is that between permeability to weak electrolytes, which is discussed in 11 pages, and that to dyes and oxidation-reduction indicators, which together receive 87 pages. Included in the latter section of the book, however, is much material that other authors with different interests would doubtless have treated in separate chapters—for example, the various theories of cell permeability, the factors which modify permeability, etc.

Almost a third of the book is devoted to the osmotic properties of cells, a field in which the senior author made one of his first scientific contributions and in

which he has retained his interest to the present day. The collection in one place of much previously scattered material pertaining to this subject will be of value to many.

A unique feature of the present volume is the attempt made in it not only to deal quantitatively with the problem of permeability but to use essentially the same type of treatment for nonelectrolytes, electrolytes, and water, respectively. This attempt, though commendable, has not, in the opinion of the reviewer, been entirely successful. A formal similarity in the units used for measurement cannot obscure certain fundamental differences in the three cases. The employment on page 11 of an equation which is admittedly theoretically unsound seems hardly justified when the problem to which it applies has been correctly treated by a number of workers.

Taken as a whole, the work is exactly what it was designed by its authors to be, namely, a stimulating discussion of certain topics in the field of cell permeability to which they have made important contributions of their own, together with much other interesting material. As such, it should have a wide usefulness.

ELEKTROPHYSIOLOGIE, VOLUME I: *ALLEGEMEINE ELEKTROPHYSIOLOGIE* VOLUME II: *SPEZIELLE ELEKTROPHYSIOLOGIE*,[1] BY HANS SCHAEFER

Reviewed by FRANK A. BROWN, JR.
Northwestern University

THESE two volumes are among numerous ones which, owing to the war, have not been generally available to American biologists until they were recently republished in this country by authority of the Alien Property Custodian. The delay has been acutely felt in this instance, since no extensive treatises upon the subject of general electrophysiology have appeared for a great number of years, and especially since the number of research papers published annually in the subject has been growing at an increasingly rapid rate. These volumes provide the physiologist with the only available modern survey of the field and with an extensive recent bibliography. Several thousand papers are listed in a painstakingly classified and indexed list of the literature. Both of the volumes were completed in Germany after the outbreak of the war, with the inevitable consequence that, while the survey of the subject is roughly complete with respect to German literature up to the approximate dates of publication, it is complete only up to about 1938–39 for non-German literature.

Unlike most similar types of monographs, these volumes contain little or no historical development of their subject. They are aimed at providing the biologist with an outline of the current general problems and theories of electrophysiology, together with an extensive list of the world literature. They are not intended to serve as comprehensive reference sources. The author confesses that under the circumstances surrounding the preparation of the books he was unable to exercise much selection in the literature lists but did try to select for consideration in the text only those pa-

[1] Vienna: Franz Deuticke, 1940–42; Ann Arbor, Mich.: J. W. Edwards, 1944. Vol. I, pp. xi+522; Vol. II, pp. ix+404. $12.00.

pers which he believed to be most significant for the development of the general theories.

Numerous sections at various points throughout the volumes are set in fine print. These include more or less extended accounts of special topics, less important material, or those parts of the subject which require some knowledge of mathematics for their understanding; hence a reader who desires only a superficial summary of the field may omit these sections.

The first volume deals with general problems in electrophysiology, i.e., those electrical phenomena which obtain more or less generally for all cells and tissues. In this volume are also included the general aspects of the electrophysiology of nerve and muscle. The volume is subdivided into the following subjects: passive electrical phenomena of living tissues; general stimulus physiology; problem of local excitation; active electrical phenomena—resting-, injury-, and action-currents; conduction of excitation from organ to organ; and electrical processes under nonphysiological conditions.

The second volume contains special electrophysiological topics and includes the following principal subjects: electrophysiology of the heart; special electrophysiology of nerve and muscle, especially human; electrophysiology of the organs of fearing and equilibrium; electrophysiology of the visual organs; electrophysiology of smell and taste; electrophysiology of the central nervous organs; special electrophysiology of the skin; and electrophysiology of electric fishes.

These chapter subjects indicate fairly well the limits of the subject as drawn by the author. Not considered are such more purely physicochemical subjects as electrophoresis and electrochemistry. Also omitted are all physiological studies in which electricity is used only as a tool or technique and such problems as the pathological effects of strong electric currents, short waves, etc. Problems in electrophysiology of a purely clinical or pathological character are not included. Nevertheless, clinical uses of many general or special electrophysiological phenomena, such as are normally associated with the heart and brain activity, etc., are amply indicated.

Both volumes are largely theoretical in content, with the first volume being, from the nature of the subject matter considered, slightly more so than the second. The factual details are brought forth primarily in support of the more generally accepted theories or those theories particularly favored by the author. Such an organization is obviously indispensable in this field. However, the facts are not interwoven with the theories which they are intended to support to such a degree that it is impossible to distinguish between the two.

Throughout the two volumes the author appears to show a good critical analysis of the general functional significance of the electrical phenomena proper. There is a constant realization of the indissociable relationship between electrical phenomena exhibited and underlying chemical changes and of the constant possibility that the electrical manifestations associated with many vital phenomena are solely nonessential by-products of indispensable chemical changes. However, it is emphasized that in certain instances, such as the electric organs of fishes, there is no doubt of the primary significance of the electrical phenomena. Also, in such processes as conduction in nerve tissue, the highly quantitative relationships existing between potential changes and states of nerve activity give

us strong reason for believing that the electrical phenomena are certainly of some direct importance here as well.

The books should be of tremendous value to physiologists, whether they are simply interested in obtaining a survey of the general field or are interested in becoming acquainted with special aspects of the subject from the standpoint of a research worker. The literature lists alone with their subject and author indexes, totaling 168 pages (nearly 20 per cent of the whole), furnish us with the best and most usable bibliography in the subject and, even apart from the valuable text discussion, would be well worth the purchase of the volumes. The figures and tables, though not so numerous as one might wish, appear well chosen. In short, Dr. Schaefer's monograph represents an excellent summary covering an area in biology that has been badly in need of review and will doubtless serve for some time to come as an invaluable source of information and as a key to the literature in this field.

INDEX TO VOLUME XVIII

ALLEE, W. C., 195, 365

AMATRUDE, CATHERINE S., 332

Antigenic relationships in the genus *Tetrahymena*, 415

ARONSON, LESTER R., 403

Atmospheric humidity, the effects of, on animal life, 103

BEACH, FRANK A., 390

BEEMAN, ELIZABETH A., 195

BEERS, C. D., 80

Behavior of melanoblasts derived from the coelomic lining in interbreed grafts of wing skin, 1

BOND, CHARLOTTE R., 52

BRADDOCK, JAMES CONGER, 176

BROOKS, MATILDA MOLDENHAUER, 431

BROOKS, S. C., 431

BROWN, FRANK A., 433

Chemistry and Physical Hormones, The, by Forest Ray Moulton (ed.), 330

CHILD, C. M., 250

Chimaeric feathers of breast-saddle origin in the Brown Leghorn capon, experimental alteration of growth rate in, 335

Cichlid fish, *Tilapia macrocephala*, influence of the stimuli provided by the male, on the spawning frequency of the female, 403

CIESLAK, EDWIN S., 229

COLE, LEON J., 167

COLLIAS, N. E., 365

CONDRIN, JOHN M., 162

COSGROVE, W. B., 425

DANIELLI, JAMES FREDERIC, 222

DAVSON, HUGH, 222

DEWEY, VIRGINIA C., 136, 415

Dominance-subordination relationship in the fish *Platypoecilus maculatus*, some aspects of the, 176

Ebony: a gene affecting the body color and fecundity of *Tribolium confusum* Duval, 35

Elektrophysiologie, Vol. I: *Allgemeine Elektrophysiologie;* Vol. II: *Spezielle Elektrophysiologie*, by Hans Schaefer, 433

Embryology of Behavior, The, by Arnold Gesell, in collaboration with Catherine S. Amatruda, 332

Excystment in the ciliate *Tillina magna*, some factors affecting, 80

FINLEY, HAROLD E., 167

Flecking in the domestic pigeon, effects of X-rays upon, 167

Frogs eggs (*Rana pipiens*), some group effects on the rate of cleavage and early development of, 16

GAUNT, ROBERT, 330

GESELL, ARNOLD, 332

GIESE, ARTHUR C., 158, 223

GINSBURG, BENSON, 35

Growth of the ciliate *Tetrahymena geleii*, mineral deficiency as a factor limiting, 425

GUHL, A. M., 365

HALL, R. P., 425

Hamster, the golden (*Cricetus auratus*): care, breeding, and growth, 52

Hibernation in the chipmunk, physiological studies on, 162

HOROWITZ, SHIRLEY, 35

JACOBS, M. H., 431

KIDDER, GEORGE W., 136, 415

LUDWIG, DANIEL, 103

MCGANN, VIRGINIA G., 415

Mating behavior in the male rat, bisexual: effects of castration and hormone administration, 390

Mating behavior and the social hierarchy in small flocks of White Leghorns, 365

MERWIN, RUTH, 16

Metabolism of *Taenia taeniaeformis*, studies on, 60

METZ, C. W., 100

Mitosis, by Franz Schrader, 100

MOULTON, FOREST RAY, 330

New Books in physiological zoölogy, 100–102; 222; 330–34; 431–33

Oxidation-reduction patterns in ciliate protozoa, 250

Paramecium, a simple method for division-rate determination in, 158

PARK, THOMAS, 35

PARPART, A. K., 222

Permeability of Living Cells, The, by S. C. Brooks and Matilda Moldenhauer Brooks, 431

Permeability of Natural Membranes, The, by Hugh Davson and James Frederic Danielli, 222

RAWLES, MARY E., 1

SCHAEFER, HANS, 433

SCHRADER, FRANZ, 100

Snake, *Thamnophis radix*, relations between the reproductive cycle and the pituitary gland in the, 299

STUART, C. A., 415

Tetrahymena, studies on the biochemistry of. III. Strain differences, 136

Thiamin on the winning of social contacts in mice, some effects of, 195

Ultraviolet radiations and life, 223

WANG, HSI, 335

WILMOTH, JAMES H., 60

WINDLE, WILLIAM F., 332

WOODWARD, ALVALYN E., 162

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